

# **North-South**



**collaboration in clinical  
pharmacological research  
of HIV treatment**

Rafaëlla L'homme

# North-South collaboration in clinical pharmacological research of HIV treatment

Een wetenschappelijke proeve  
op het gebied van de Medische Wetenschappen

## Proefschrift

ter verkrijging van de graad van doctor  
aan de Radboud Universiteit Nijmegen  
op gezag van de Rector Magnificus prof. mr. S.C.J.J. Kortmann,  
volgens besluit van het College van Decanen,  
in het openbaar te verdedigen op woensdag 22 oktober 2008  
om 15.30 uur precies

door

**Rafaëlla Francisca Anna L'homme**

geboren op 1 juli 1978 te Heerlen

Publication of this thesis was financially supported by:  
Stichting Klinisch Farmaceutische Dienstverlening, Nijmegen

Design cover and lay out by In Zicht Grafisch Ontwerp, Arnhem  
Printed and bound by PrintPartners Ipskamp, Enschede

ISBN 978-90-9023354-3

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**Promotor**

Prof. dr. Y.A. Hekster

**Co-promotor**

Dr. D.M. Burger

**Manuscriptcommissie**

Prof. dr. R. de Groot (voorzitter)

Prof. dr. J.R.B.J. Brouwers, Rijksuniversiteit Groningen

Prof. dr. J.M.A. Lange, Academisch Medisch Centrum Amsterdam

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**Introduction**

## AIDS and HIV infection

In 1981, the acquired immunodeficiency syndrome (AIDS) was described for the first time in previously healthy homosexual men [1]. Two years later, the human immunodeficiency virus (HIV) was discovered as the cause of AIDS [2]. HIV belongs to the class of retroviruses, using the enzyme reverse transcriptase to convert viral RNA into DNA [3]. For the process of replication, the virus needs the CD4 cells of the human immune system, leading to destruction of the human immune response [4]. HIV can be transmitted through unprotected sexual intercourse, mother-to-child transmission, transfusion of contaminated blood or the use of contaminated injection needles.

## Antiretroviral drugs

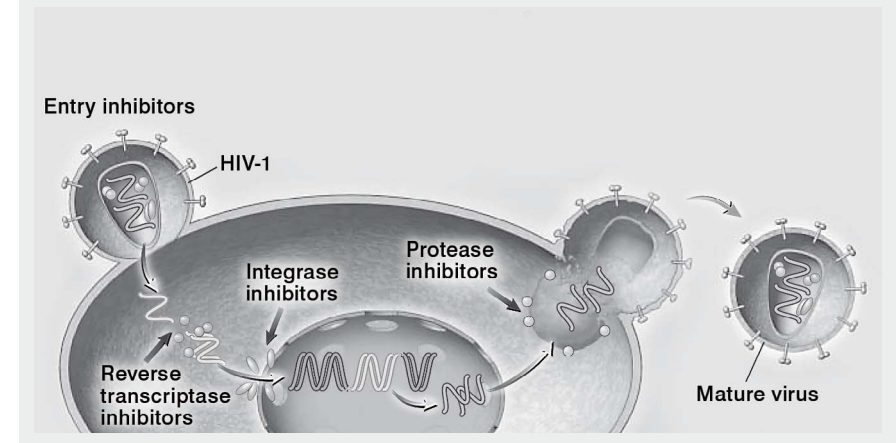
Survival of HIV-infected subjects can be prolonged by treatment with antiretroviral drugs [5]. To achieve highly active antiretroviral therapy (HAART), at least three antiretroviral drugs from at least 2 different classes should be combined. The five classes of antiretroviral drugs are shown in Table 1. Each of the classes has a unique target in the life cycle of the virus (Figure 1).

**Table 1** Overview of antiretroviral drug classes.

NRTIs	NNRTIs	Protease inhibitors	Entry inhibitors	Integrase inhibitors
Abacavir	Delavirdine	Atazanavir	Enfuvirtide	Raltegravir
Didanosine	Efavirenz	Darunavir	Maraviroc	
Emtricitabine	Nevirapine	Fosamprenavir		
Lamivudine		Indinavir		
Tenofovir		Lopinavir		
Stavudine		Nelfinavir		
Zalcitabine		Ritonavir		
Zidovudine		Saquinavir		
		Tipranavir		

NRTIs: nucleoside reverse transcriptase inhibitors  
 NNRTIs: non-nucleoside reverse transcriptase inhibitors

**Figure 1** Targets of antiretroviral drug classes.



## Nucleoside reverse transcriptase inhibitors

Nucleoside reverse transcriptase inhibitors (NRTIs) inhibit the replication of HIV by inhibition of the enzyme reverse transcriptase. NRTIs are prodrugs that require intracellular phosphorylation to the active tri-phosphate metabolites [6]. Tenofovir is the only nucleotide analogue that must be di-phosphorylated as it already contains one phosphate group [7]. The first drug to treat HIV, zidovudine, was introduced in 1987 and belongs to the NRTI class [8].

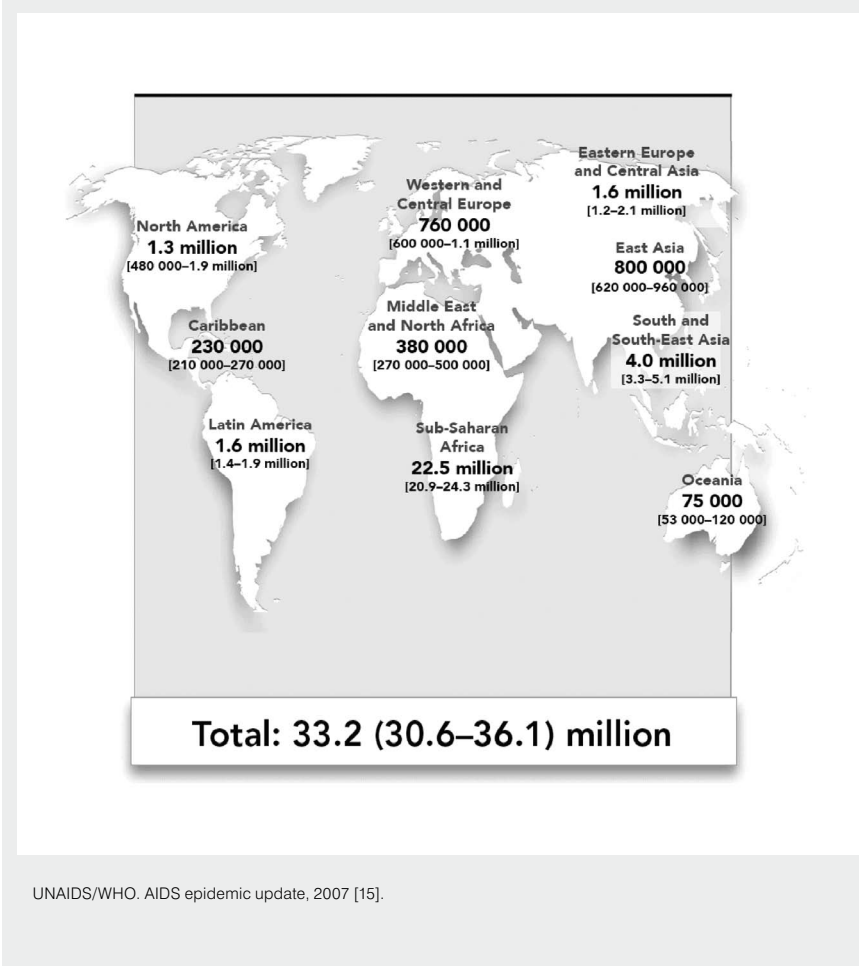
## Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) inhibit the replication of HIV by induction of allosteric changes to the enzyme reverse transcriptase. In contrast to NRTIs, NNRTIs do not require phosphorylation to become active [9]. Nevirapine was the first NNRTI to be approved in 1996 [10].

## Protease inhibitors

Protease inhibitors inhibit the reproduction of HIV by inhibition of the enzyme protease. Saquinavir, indinavir and ritonavir were the first protease inhibitors to be approved in 1996 [11].

**Figure 2** Adults and children estimated to be living with HIV in 2007.



## Entry inhibitors

Entry inhibitors inhibit the reproduction of HIV by inhibition of HIV entry into the cell. A subclass of fusion inhibitors is active through inhibition of fusion between HIV and cellular membranes [12]. The first and only fusion inhibitor, enfuvirtide, was approved in 2003 [13]. Maraviroc is the first entry inhibitor of a new subclass of CCR5 antagonists that was recently approved [14]. CCR5 antagonists inhibit the entry of HIV into the cell by inhibition of binding to the co-receptor CCR5.

## Integrase inhibitors

A new class of integrase inhibitors can inhibit the reproduction of HIV by inhibition of the enzyme integrase. Raltegravir is the first integrase inhibitor that was recently approved [14].

## Epidemiology of HIV infection

Global estimates from the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) show that 33.2 million people, of whom 2.5 million children, were living with HIV at the end of 2007 [15]. The numbers of infected adults and children per region are shown in Figure 2. The region most affected by the AIDS epidemic remains Sub-Saharan Africa, where 68% of the adults and nearly 90% of the children infected with HIV live. Of all AIDS deaths in 2007, 76% occurred in Sub-Saharan Africa, illustrating the unmet need for HIV treatment in resource-limited countries.

## Clinical pharmacological research of HIV treatment

Although challenging, clinical pharmacological research of HIV treatment in resource-limited countries can be facilitated by sponsorships and North-South collaboration between partners from the developed and developing world. Most of the research in this thesis was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP) and by the Poverty Related Infection Oriented Research (PRIOR) network that was established by a grant from the Netherlands Foundation for the Advancement of Tropical Research (NWO-WOTRO) and the Netherlands Foundation for Health Research and Development (ZonMw). The North-South collaboration consisted of two European institutes (Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands and the Medical Research Council, Clinical Trials Unit, London, United Kingdom) and two African institutes (University Teaching Hospital, Lusaka, Zambia and Kilimanjaro Christian Medical Centre, Moshi, Tanzania). This thesis focuses on clinical pharmacology in HIV-infected children (**Part I**), HIV-infected adults (**Part II**) and HIV-TB co-infection (**Part III**).

## I. Clinical pharmacology in HIV-infected children

A major barrier to scaling up HIV treatment for children in resource-limited areas of the world has been the lack of affordable, accessible and appropriate paediatric

antiretroviral drug formulations [16]. This has led to the pragmatic approach of prescribing generic adult fixed dose combination (FDC) tablets of nevirapine, stavudine and lamivudine to children. In addition to difficulties with accurate cutting and possible unequal distribution of drugs within tablets, a major problem is that the drug ratios are designed for adult dosing. Pharmacologically speaking, HIV-infected children cannot simply be regarded as small adults. Pharmacokinetics of antiretroviral drugs are highly variable in the paediatric population as children mature and grow rapidly and individually until they are adults [17]. For example, (young) children metabolize the NNRTI nevirapine more rapidly than adults. **Chapter 1** focuses on general characteristics related to pharmacokinetic processes in HIV-infected children. **Chapter 2** describes the exposure to nevirapine in HIV-infected Malawian and Zambian children treated with divided adult FDC tablets.

Recently, paediatric-friendly generic FDC tablets were developed for HIV-infected children with higher nevirapine to stavudine and lamivudine ratios than adult tablets in accordance with paediatric dose recommendations. We performed an independent pilot bioequivalence study of these paediatric FDC tablets in comparison to the branded products (**Chapter 3**). Subsequently, we investigated whether the antiretroviral drug ratio in the paediatric FDC tablets results in optimal exposure of nevirapine, stavudine and lamivudine in a target population of Zambian HIV-infected children (**Chapter 4**).

## II. Clinical pharmacology in HIV-infected adults

The access to HIV treatment for adults in resource-poor countries has improved in the past few years, mainly due to global efforts such as the “3 by 5” initiative of WHO [18]. Most of the generic regimens that have become available are nevirapine-based. A disadvantage of NNRTI drugs such as nevirapine is the low genetic barrier for the development of resistance [19]. Patients at risk for virological failure are those with subtherapeutic plasma concentrations of nevirapine [20-22]. Therapeutic Drug Monitoring (TDM) is a well-known tool for the optimization of nevirapine dosing in the developed world [22], but is not often performed in resource-limited settings due to the lack of simple and affordable assays to determine the exposure to nevirapine. **Chapter 5** describes the validation of a simple and economical thin

layer chromatography method for semi-quantitative detection of nevirapine in saliva of HIV-infected adults.

Complexity and costs of several effective strategies to prevent mother-to-child transmission (MTCT) of HIV have limited large-scale introduction in low-income countries. Administration of a single dose nevirapine to the mother shortly before delivery and to the newborn after birth was found to be a simple and cost-effective strategy to reduce the risk of MTCT in low-income countries by 50% [23]. A major disadvantage is the development of resistance in up to 70% of women [24], which may diminish the effectiveness of nevirapine when a mother becomes pregnant again or acquires an indication for nevirapine-based HAART in the future [25]. It is likely that nevirapine resistance develops due to the combination of a low genetic barrier to resistance [19] and a long half-life [26]. The risk for the development of resistance is increased when subtherapeutic nevirapine concentrations remain in the blood for a long period of time. A short-course of an enzyme inducer may reduce resistance development by increasing metabolism and decreasing half-life of nevirapine. In **Chapter 6**, a pilot study investigating the effect of enzyme inducers on the elimination half-life of nevirapine is presented. The study was conducted in the Netherlands as prelude to a study in Tanzania to test a suitable intervention for its ability to reduce the development of nevirapine resistance.

## III. Clinical pharmacology in HIV-TB co-infection

Tuberculosis (TB) is a common opportunistic infection in HIV-infected subjects, particularly in developing countries [27]. Co-administration of HIV and TB treatment is frequently indicated. Standard of care treatment regimens for TB include rifampicin, which is a strong inducer of cytochrome P450 enzymes, resulting in drug-drug interactions with antiretroviral therapy [28]. Patients on rifampicin are preferably treated with efavirenz-based HAART [27], which does not lead to a clinically relevant interaction [29, 30]. Although rifampicin reduces nevirapine exposure by 20-55% [31], nevirapine is an alternative to efavirenz among non-Caucasian patients [32, 33].

Second-line treatment for patients who have failed NNRTI-based regimens does often include protease inhibitors [27]. Rifampicin, however, causes large decreases



in the exposure to protease inhibitors [34-37]. We previously demonstrated that increased doses of lopinavir/ritonavir soft gel capsules (SGCs) compensate for the interaction with rifampicin [34]. Recently, the SGC formulation was replaced by a new heat-stable tablet formulation that exhibits slightly higher bioavailability irrespective of food intake. This has been a major improvement, especially for resource-limited settings where there may be a lack of options for refrigeration or food. Data from the interaction study with rifampicin and the SGC formulation of lopinavir/ritonavir cannot simply be extrapolated to the new tablet formulation. **Chapter 7** describes the subsequent study in healthy volunteers evaluating the pharmacokinetics of two adjusted dose regimens of lopinavir/ritonavir tablets in combination with rifampicin. Clinical experience with the combined use of lopinavir/ritonavir and rifampicin in the Netherlands is described in **Chapter 8**.

## Objectives of this thesis

This thesis presents the first output of a North-South collaboration in clinical pharmacological research of HIV treatment. **Part I** deals with the optimization of paediatric regimens for the scale-up of HIV treatment in resource-limited countries. **Part II** focuses on TDM in HIV-infected adults and the optimization of a cost-effective strategy for the reduction of MTCT in low-income countries. In **Part III**, the challenges of combined treatment with lopinavir/ritonavir and rifampicin for HIV-TB co-infection are described. Finally, a General discussion is presented.

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## **Part I**

Clinical pharmacology  
in HIV-infected children

A stylized illustration of several hands reaching upwards from the bottom of the frame towards the top. The hands are rendered in various shades of gray, with the central hand being white. The background is a dark gray gradient with a pattern of small, light gray circles that become more dense towards the right side.

# Chapter 1

Children with HIV are not small adults: what is different in pharmacology?

Rafaëlla L'homme<sup>1,2</sup>, Adilia Warris<sup>2,3</sup>, Diana Gibb<sup>4</sup>, David Burger<sup>1,2</sup>

<sup>1</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands; <sup>3</sup> Department of Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>4</sup> Medical Research Council, Clinical Trials Unit, London, UK

*Current Opinion in HIV and AIDS* 2007; 2:405-9

## Purpose of review

Pharmacokinetics of antiretroviral drugs are highly variable among HIV-infected children. This review describes pharmacokinetic processes in children and recent pharmacokinetic data in children with HIV. The general lack of pharmacokinetic data and the potential role of therapeutic drug monitoring (TDM) is being discussed.

## Recent findings

It was unexpectedly found that exposure to lopinavir is decreased in the first 6 months of life. Recent findings of subtherapeutic efavirenz concentrations in children suggest that pediatric dose recommendations should be re-evaluated. In addition, recommended dosing of lamivudine leads to lower exposure in children younger than 6 years of age. Preliminary results of pediatric fixed-dose combination (FDC) tablets for HIV-infected children with a higher nevirapine to stavudine and lamivudine ratio than adult FDCs suggest adequate drug exposure. As an alternative to plasma sampling, concentrations of nevirapine can be determined in saliva.

## Summary

There is a shortage of pharmacokinetic data in the highly variable population of HIV-infected children. Selected pharmacology studies should be undertaken to improve pediatric dose guidance of existing antiretroviral drugs. TDM is a useful tool to optimize treatment in HIV-infected children. However, more data are needed to establish child-specific reference values.

## Keywords

children, HIV, pharmacokinetics

## Introduction

HIV-infected children cannot simply be regarded as small adults, pharmacologically speaking. Pharmacokinetics of antiretroviral drugs are highly variable in the pediatric population as children mature and grow rapidly and individually until they are adults. This brief review focuses on general characteristics relating to the processes in pharmacokinetics (absorption, distribution, metabolism, excretion) in the pediatric population, followed by recent examples of antiretroviral drug pharmacokinetics in HIV-infected children. In addition, practical dosing issues of antiretroviral drugs in children are taken into account. Finally, the role of therapeutic drug monitoring for HIV-infected children is being discussed.

## Absorption

The gastrointestinal absorption of oral drugs in children is different from that in adults, because of developmental changes of the gastrointestinal tract. During the first 2 years of life, the pH in the stomach of children is high compared with the pH in adults [1\*\*]. Gastric emptying time is delayed in neonates, whereas in infants and older children gastric emptying time is more rapid than in adults [2]. Similarly, intestinal motility is reduced in neonates and increased in older infants [1\*\*]. Furthermore, intestinal drug absorption in neonates may be influenced by increased permeability of the mucosa, immature biliary function, reduced first-pass metabolism, maturation of carrier mechanisms and variable microbial colonisation [1\*\*].

Food restrictions to optimize absorption of some antiretroviral drugs can be problematic in young HIV-infected children who need regular feeding and may not tolerate high-fat meals [2]. The authors of one study supposed that the much higher apparent nelfinavir plasma clearance and volume of distribution found in children below the age of 2 years was due to a decrease in bioavailability [3\*]. These results were in agreement with our previous finding that children below 2 years tend to be at higher risk for low plasma concentrations of nelfinavir [4]. The reduced bioavailability in infants can be explained by differences in diet between young infants and older children, as the bioavailability of nelfinavir is influenced by the quantity and type of food with which it is administered [3\*, 5]. Moreover,

bioavailability of weakly acidic compounds like nelfinavir may be reduced in the first 2 years of life due to a higher pH in the stomach [3\*]. Atazanavir and indinavir are 2 other examples of antiretroviral drugs that require an acidic gastric medium for adequate absorption [6]. Therefore, absorption of atazanavir and indinavir may also be reduced in the first 2 years of life, when gastric pH is slowly declining towards adult levels. This effect theoretically could abate with concurrent ritonavir use [6]. While there are insufficient data to recommend atazanavir with or without ritonavir or indinavir plus ritonavir in young children [7\*\*], studies have been presented on the use of these antiretroviral drugs in older children. Recently, first data were reported on the pediatric use of ritonavir-boosted atazanavir by 23 children and adolescents with a median age of 16 years (range, 10-19 years) [8\*]. The adult dose of 300 mg atazanavir and 100 mg ritonavir in children weighing more than 50 kg and the reduced dose of 200 mg atazanavir and 100 mg ritonavir in children weighing less than 50 kg, grossly gave a satisfactory plasma concentration at random timing in children with good adherence. Nevertheless, virological failure was observed in one third of the children identified as good compliers; the authors claim that this may have been caused by the insufficient antiviral potency of the nucleoside reverse transcriptase inhibitors (NRTIs) that were used as backbone [8\*]. Reduced dosages of indinavir (220-300 mg/m<sup>2</sup>), when boosting with either 100 mg or with full ritonavir dosages appeared to provide adequate indinavir concentrations in most out of 19 Thai children with median ages of 9.7 and 11.3 years, respectively [9]. It is important to keep in mind that the available pharmacokinetic data for atazanavir and indinavir mentioned above are mainly from older children, in whom the level of gastric pH is not expected to influence absorption.

## Distribution

The distribution of drugs in the body changes during childhood, because of age-specific differences in water content and plasma protein concentrations. The volume of total body water and extracellular fluid is high in young infants [1\*\*]. Drugs with a small volume of distribution (Vd <0.4 L/kg in adults) distribute over the extracellular fluid, while drugs with a large Vd (>0.6 L/kg in adults) are extensively tissue bound [1\*\*]. Since body surface area (BSA) correlates with extracellular fluid volume, BSA may be used to predict the dose of drugs with a small Vd in young

children. Doses of drugs with a large Vd can be based on body weight, because tissue binding capacity and Vd in children are similar to those in adults [1\*\*]. The fact that protein binding tends to be reduced in neonates may be relevant for drugs with high protein binding capacity in combination with a small Vd. An increase in the unbound drug fraction may result in a larger Vd and increased efficacy and toxicity on short-term. However, stabilization of the unbound drug concentration may be expected due to enhanced clearance of the drug and therefore dose adjustments do not necessarily seem to be indicated [1\*\*].

Enfuvirtide, the first fusion inhibitor for the treatment of HIV-infection, is administered subcutaneously and exhibits a small Vd, low systemic clearance and high plasma protein binding [10]. Body weight adjusted dosing (2.0 mg/kg bid) in 25 HIV-infected children between 5 and 16 years of age resulted in enfuvirtide exposure similar to that obtained in HIV-infected adults receiving a dosage of 90 mg bid [11]. Recently, it was confirmed that pediatric enfuvirtide dosing per body weight is appropriate in children from 6 years of age as enfuvirtide exposure was not affected by age [12\*]. No data are available regarding the use of enfuvirtide in neonates. Another example of a drug with high protein binding capacity in combination with a small Vd is the relatively new antiretroviral tipranavir. The pharmacokinetic profile of tipranavir in pediatric patients is currently under investigation [13].

## Metabolism

The rate of drug metabolism in children is influenced by changes in hepatic enzyme activity. Drugs can be metabolized by hepatic enzymes involved in phase 1 (oxidation, reduction and hydrolysis) and phase 2 (conjugation) reactions. Cytochrome (CYP) P450 is an important mixed-function oxidase system with reduced activity in neonates. This explains the fact that most drugs have a prolonged elimination half-life in neonates. The elimination half-life of most substrates approaches adult levels at the age of 2 to 6 months, when a significant amount and activity of enzymes is present [1\*\*]. Activity of liver enzymes exceeds adult levels at 1 to 4 years and declines to adult levels at the end of puberty [2]. Below the age of 2 months it seems wise to start with a very low dose and modify subsequent doses based on response, adverse events and if possible drug

concentrations. Between 2 and 6 months of age careful dosing of drugs based on body weight appears to be appropriate [1\*\*]. After maturation of liver enzymes at the age of 6 months, metabolism is mainly dependent on liver growth, except for metabolism by the enzymes glucuronosyltransferase (UGT) and CYP2D6. As liver volume correlates well with BSA, it seems reasonable to base drug doses in children from the age of half a year on BSA as long as UGT and CYP2D6 are not involved in the pathway of metabolism [1\*\*].

Metabolism of antiretroviral drugs from the protease inhibitor (PI) and non nucleoside reverse transcriptase inhibitor (NNRTI) classes in particular varies markedly with age in children and is also more variable between children of the same age and size compared to adults. In this paragraph, the focus will be on the PI-combination lopinavir/ritonavir and the NNRTIs nevirapine and efavirenz as these are the preferred options to be given in combination with 2 NRTIs as initial therapy for HIV-infected children. Metabolism of lopinavir is predominantly mediated by hepatic CYP3A4. Ritonavir inhibits the activity of CYP3A4 resulting in boosted plasma lopinavir concentrations [14]. Jullien et al have recently shown that lopinavir pharmacokinetics vary greatly during childhood and that children below the age of 6 months and boys above the age of 12 years may have decreased levels of exposure to lopinavir [15\*\*]. The increased ability to eliminate lopinavir in neonates was surprising when looking at the pharmacokinetic process of metabolism alone, as the activity of CYP3A4 is known to be markedly reduced in the first weeks of life [15\*\*]. However, when taking into account the pharmacokinetic processes of absorption and distribution, a decrease in the pharmacokinetic enhancement provided by ritonavir was found to be the most likely explanation [15\*\*]. This example shows us the importance of looking at all aspects of the pharmacokinetic process and the actual testing of our hypotheses in the target population of HIV-infected children. Once daily dosing of lopinavir/ritonavir (460/115 mg/m<sup>2</sup>) in 19 HIV-infected children between 1.4 and 12.9 years resulted in mean pharmacokinetic parameters comparable to once daily dosing (800/200 mg) in adults, although the variability in trough levels was much higher in children [16\*\*]. Subtherapeutic trough levels were more often experienced in younger children [16\*\*]. In 2003, virologic responses in 74 HIV-infected children (1.6 months to 14.5 years) supported the use of nevirapine doses greater than 300 mg/m<sup>2</sup>/day, which was higher than recommended by the manufacturers at the time of publication [17]. Recently, in 127

African children aged 8 months to 18 years, subtherapeutic nevirapine concentrations were only observed in 3% of those prescribed  $\geq 300$  mg/m<sup>2</sup>/day compared to 23% of those prescribed  $< 300$  mg/m<sup>2</sup>/day [18\*\*]. Preliminary results in Zambian children of at least 6 months that were dosed according to this 300 mg/m<sup>2</sup>/day cut-off were promising as few subtherapeutic concentrations were observed [19]. With this knowledge, it is inappropriate to support dosing of nevirapine below 300 mg/m<sup>2</sup>/day in HIV-infected children at this stage. Ren et al have recently presented data on efavirenz concentrations in children with and without the anti-tuberculous enzyme inducer rifampin [20]. Standard recommended doses were used. Rifampin did not significantly reduce efavirenz concentrations. However, the fact that 50% of the children had a subtherapeutic efavirenz concentration, suggests that recommendations for efavirenz doses should be re-evaluated. An example of reduced first-pass metabolism of the NRTI zidovudine in neonates was published longer ago. After an oral dose of zidovudine, bioavailability was 89% in the first 2 weeks of life and 61% in children that were older than 2 weeks [21].

## Excretion

Drug excretion in children is influenced by maturation of the kidney function. Glomerular filtration (GFR) and active tubular secretion (AS) are reduced in neonates and rise to adult values at about 3 and 7 months of age, respectively [22]. In older children, GFR and AS may actually exceed adult values [22]. Peak renal functions occur between the ages of 3 and 5 years [2]. Logically, drug dosing in children should be based on GFR if excretion by GFR is most significant and should be based on AS if excretion by AS is most significant [1\*\*]. In the first week of life GFR may be based on an inert marker or gestational age, while after the first week of life plasma creatinine may be used as a marker for GFR [1\*\*]. In children above 2 years without renal insufficiency drug doses can be based on BSA, since there is a good correlation with GFR [1\*\*].

The NRTI lamivudine is preliminarily eliminated by renal excretion of non-metabolized drug. The renal clearance of lamivudine was found to be greater than the glomerular filtration rate, which implies that lamivudine is also actively secreted into the renal tube [23]. A higher dose of lamivudine is recommended in children (4 mg/kg bid)

compared to adults (150 mg:  $\pm$  2 mg/kg bid). We have recently demonstrated that recommended dosing of lamivudine in a group of 51 children between 1.7 and 18 years leads to lower plasma exposure and maximum concentration of lamivudine in children younger than 6 years of age when compared with older children or adults [24\*\*]. Part of the explanation could be the use of oral solution in younger children and tablets in older children, as the bioavailability of the solution may be less in children. Another reasonable explanation could be enhanced renal clearance in children between 1.7 and 6 years of age compared to older children.

## Practical dosing issues

Adult antiretroviral formulations do not allow doses to be easily adjusted in small increments as the child grows. The development of pediatric friendly liquid formulations of individual antiretroviral drugs has been a good solution for the treatment of HIV-infected children in the developed world. In contrast, liquid formulations are generally no option for children in resource limited settings, where liquids are too expensive and too difficult to transport and store due to large volumes and short shelf-lives. The mainstay of first-line therapy for HIV-infected adults in resource-limited settings is a fixed-dose combination (FDC) of stavudine, lamivudine and nevirapine. Recent findings demonstrate that it is difficult to achieve adequate nevirapine levels with divided adult FDC tablets in young children without the risk of overdosing with stavudine [18\*\*]. However, newly developed small, crushable, dispersible and scored FDC tablets for HIV-infected children with a higher proportion of nevirapine are currently undergoing clinical investigation and preliminary results suggest adequate exposure of all three antiretrovirals over the entire range of ages [19]. Development of solid pediatric formulations containing a PI may be problematic as minimum tablet sizes are expected to be too big for pediatric use.

## Role of Therapeutic Drug Monitoring

Treatment of HIV-infected children may be considered as an indication for therapeutic drug monitoring (TDM), because of high inter- and inpatient variability of antiretroviral drug concentrations [25]. In HIV-infected adults, plasma drug

concentrations of PIs and NNRTIs are better predictors of antiretroviral response or toxicity than drug dose is [26]. This is expected to be even more evident in HIV-infected children where guidelines may advise inconsistent categories or wide ranges of drug doses due to the lack of sufficient pharmacokinetic data. For example, the US Food and Drug Administration (FDA) approved lopinavir/ritonavir for HIV-infected children from the age of 6 months even though the dose validation in the lowest weight group was limited to a small number of children, while the European Medicines Agency (EMA) approved the combination only for children of at least 2 years of age [16\*\*]. It would be helpful to have some guidance on the minimum number of children per weight group that should be used in pharmacokinetic studies to register antiretroviral drugs for the use in children. Since little or no target drug levels are available for children, antiretroviral drug concentrations in children are targeted to adult reference values [25]. Adult references may be insufficient for children because of the unique features of HIV-infection in children such as immature immune systems and high viral loads or may be too high in case of increased sensitivity of children for side effects [2]. In addition to optimization of antiretroviral therapy dosage for children, TDM may be used as a direct and objective instrument to measure non-adherence, which is a substantial problem in pediatric HIV-infected children [2]. A new interest of research is the development of noninvasive, reliable and cost-effective methods for application of TDM in HIV-infected children. As an alternative to plasma sampling, concentrations may be determined in saliva for antiretroviral drugs with low protein binding like nevirapine [27\*].

## Conclusion

Due to the shortage of pharmacokinetic data in the highly variable population of HIV-infected children, antiretroviral drug dosing remains complicated. Selected pharmacology studies should be undertaken to improve pediatric dose guidance of existing antiretroviral drugs. Furthermore, the new European regulation for pediatric drugs will lead to an obligation for pediatric research for every new drug developed for adults and having a potential use for children, in exchange for a six month extension of the supplementary protection certificate. TDM can be used to optimize treatment for the individual HIV-infected child. However, more data are needed to establish child-specific reference values.



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A stylized illustration featuring several hands of varying shades of gray reaching upwards from the bottom. The background is filled with a pattern of small, light gray circles. The hands are positioned at different heights and angles, creating a sense of movement and reaching.

## Chapter 2

**Nevirapine concentrations in  
HIV-infected children treated  
with divided fixed-dose  
combination antiretroviral  
tablets in Malawi and Zambia**

Jane Ellis<sup>1</sup>, Rafaëlla L'homme<sup>2,3</sup>, Fiona Ewings<sup>4</sup>, Veronica Mulenga<sup>5</sup>, Frank Bell<sup>1</sup>, Regina Chileshe<sup>5</sup>, Elizabeth Molyneux<sup>1</sup>, Julia Abernethy<sup>4</sup>, Joep van Oosterhout<sup>6</sup>, Chifumbe Chintu<sup>5</sup>, Sarah Walker<sup>4</sup>, Diana Gibb<sup>4</sup>, David Burger<sup>2,3</sup>

<sup>1</sup> Department of Paediatrics, College of Medicine, Blantyre, Malawi; <sup>2</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands;

<sup>3</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands;

<sup>4</sup> Medical Research Council, Clinical Trials Unit, London, UK; <sup>5</sup> Department of Paediatrics and Child Health, University Teaching Hospital, Lusaka, Zambia; <sup>6</sup> Department of Medicine, College of Medicine, Blantyre, Malawi

## Abstract

**Objective:** To investigate nevirapine concentrations in African HIV-infected children receiving divided Triomune® tablets (stavudine+lamivudine+nevirapine).

**Design:** Cross-sectional study.

**Methods:** Steady-state plasma nevirapine concentrations were determined in Malawian and Zambian children aged 8 months–18 years receiving Triomune® in routine outpatient settings. Predictors from height-for-age, BMI-for-age, age, sex, post-dose sampling time and dose/m<sup>2</sup>/day were investigated using centre-stratified regression with backwards elimination ( $p < 0.1$ ).

**Results:** Of the 71 Malawian and 56 Zambian children (median age 8.4 versus 8.5 years, height-for-age -3.15 versus -1.84 respectively), only 1 (3%) of those prescribed  $\geq 300$  mg/m<sup>2</sup>/day nevirapine had subtherapeutic concentrations ( $< 3.0$  mg/L) compared with 22 (23%) of those prescribed  $< 300$  mg/m<sup>2</sup>/day; most children with subtherapeutic nevirapine concentrations were taking half or quarter Triomune® tablets. Lower nevirapine concentrations were independently associated with lower height-for-age (indicating stunting) (+0.37 mg/L per unit higher [95% CI -0.003, +0.74],  $p = 0.05$ ), lower prescribed dose/m<sup>2</sup> (+0.89 mg/L per 50 mg/m<sup>2</sup> higher [+0.32, +1.46],  $p = 0.002$ ) and higher BMI-for-age (indicating lack of wasting) (-0.42 mg/L per unit higher [-0.80, -0.04],  $p = 0.03$ ).

**Conclusions:** Currently available adult fixed dose combination tablets are not well suited for children, particularly at younger ages: Triomune® 30 is preferable to Triomune® 40 because of the higher dose of nevirapine relative to stavudine. Further research is required to confirm that concentrations may be reduced in stunted but increased in wasted children. Development of appropriate paediatric fixed dose combination tablets is essential if antiretroviral therapy is to be made widely available to children in resource-limited settings.

## Introduction

There are 2.2 million HIV-infected children in sub-Saharan Africa of whom 370,000 are in urgent need of antiretroviral therapy (ART) [1, 2]. Operational challenges to scaling up ART for children include a lack of HIV diagnostic and monitoring tests for infants and limited expertise of health personnel in caring for HIV-infected children receiving ART. However a major obstacle in resource-limited settings is lack of affordable, accessible and appropriate paediatric antiretroviral drug formulations [3].

In order to provide ART to HIV-infected children, several countries have taken the pragmatic approach of prescribing divided adult fixed dose combination (FDC) antiretroviral tablets of Triomune® 30 or 40 (30 or 40 mg stavudine, 150 mg lamivudine, 200 mg nevirapine) to children. In addition to difficulties with accurate cutting and possible unequal distribution of drugs within tablets, a major problem with such an approach is that the ratios of drugs are designed for adult dosing. Since the rate at which children metabolise drugs is different to that of adults, and this may also vary with age, the ratios required will be different, particularly where dosing recommendations for children are based on surface area, as for nevirapine [4].

There are limited pharmacokinetic and dosing data on nevirapine as part of a FDC in children [5, 6] and how these may vary with ethnicity and degree of malnutrition. Here we aimed to determine steady state plasma concentrations of nevirapine in HIV-infected Malawian and Zambian children receiving divided adult fixed dose combination tablets of Triomune® 30 or 40, many of whom were malnourished.

## Methods

### Study population

This cross-sectional study took place in the paediatric ART clinics of Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi and the University Teaching Hospital (UTH), Lusaka, Zambia between December 2004 and June 2005. HIV-infected children aged 8 months to 18 years who had received Triomune® 30 or 40 for at least a month were enrolled. Ethical approval was obtained and written informed consent was sought from parents or guardians, and assent from children

**Table 1** Baseline characteristics by centre.

	QECH (n=71)	UTH (n=56)	p
Age, years	8.4 (4.5,11.9)	8.5 (7.0,12.6)	0.22
Age 0.5-3 years old	17 (24%)	0 (0%)	<0.001
Sex, female	31 (44%)	30 (54%)	0.27
Weight, kg	19.1 (12.4,24.9)	21.5 (18.3,32.0)	0.01
Height, cm	111 (95,129)	121 (109,135)	0.01
BMI, kg/m <sup>2</sup>	15.4 (14.5,16.5)	15.7 (14.5,17.2)	0.65
Weight-for-age z-score	-2.76 (-3.74,-1.79)	-1.77 (-2.40,-0.99)	0.0001
Height-for-age z-score	-3.15 (-3.82,-2.42)	-1.84 (-2.98,-1.17)	0.0001
BMI-for-age z-score	-0.89 (-1.64,0.07)	-0.50 (-1.63,0.27)	0.55
Total daily prescribed nevirapine dose			
mg/kg	9.8 (9.1,10.5)	11.1 (9.6,12.3)	<0.0001
mg/m <sup>2</sup>	243 (217,270)	288 (250,344)	<0.0001
Total daily prescribed lamivudine dose			
mg/kg	7.3 (6.9,7.9)	8.3 (7.2,9.2)	<0.0001
Total daily prescribed stavudine dose *			
mg/kg	2.0 (1.8,2.1)	1.4 (1.7,1.8)	<0.0001
Time between starting ART and sampling (months) †	5.9 (3.2,8.1)	8.7 (5.5,17)	0.0004
Time between last dose and sampling, hours ‡	8.9 (8.3,9.6)	3.5 (2.5,4.2)	<0.0001

Values given are median (IQR) and ranksum p-values comparing centres for continuous variables and number (%) and p-value comparing centres using chi-squared test for categorical variables.  
\* Triomune® 40 at QECH, Triomune® 30 at UTH.  
† Date of starting ART missing for one UTH child.  
‡ Sampling time missing for 7 UTH children.

where appropriate. At QECH, HIV-infected children were prescribed Triomune® 40 tablets (the only product available in Malawi) according to body weight as per national guidelines [7], either whole (containing 200 mg nevirapine) or divided into quarter (50 mg nevirapine), half (100 mg nevirapine) or three-quarter (150 mg nevirapine) parts. At UTH, children >10kg only, in long term follow-up of a previous trial of cotrimoxazole prophylaxis [8], were prescribed half or whole Triomune® 30 tablets according to surface area as per WHO guidelines [9]; children weighing <10kg received liquids and were excluded from this study. Triomune® tablets are

not scored; the pills were split at home at both centres, though QECH provided pill cutters for this purpose. Nevirapine was dose escalated in both centres, with all children receiving half nevirapine dose for 2 weeks before proceeding to full nevirapine dose. During this dose escalation phase, the children at QECH received Triomune® 40 once daily with Lamivir-S® (40 mg stavudine, 150 mg lamivudine) once daily. At UTH, the children received Triomune® 30 once daily only. Once on full dose, the majority of children received Triomune® twice daily.

Single steady state blood samples were collected at routine clinic visits, at variable times post-dose, and plasma was separated and stored at -70°C prior to transportation to the Netherlands. Nevirapine concentrations were assayed at Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, using validated HPLC [10]. Data were collected on demographics, growth parameters, last drug dose, timing of sample compared to last intake and co-medication.

Statistical methods

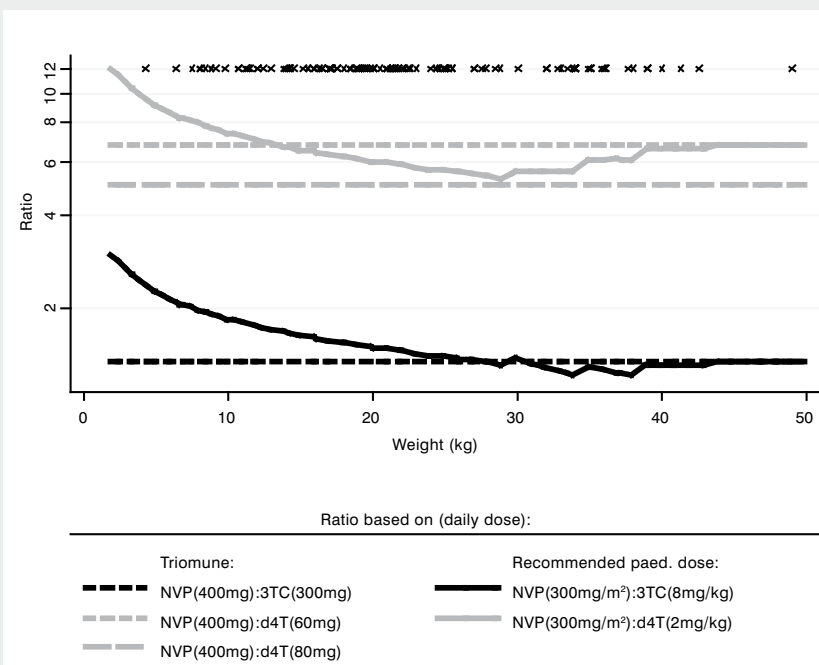
We used height-for-age to capture stunting and BMI-for-age as a pseudo weight-for-height score to capture wasting, calculated using the 1990 British Growth Charts [11] in Stata 9 [12]. CDC weight-for-height [13] could not be used as 50% of the children were outside the height range.

We estimated the effects of height-for-age, BMI-for-age, age, sex, time between last dose and sampling and dose/m<sup>2</sup>/day using linear regression of nevirapine concentration and logistic regression of therapeutic nevirapine concentration (≥ 3.0 mg/L or not, chosen as a consensus to be that best reflective of clinical practice [14-16]), stratifying for centre using meta-analysis methods with fixed effects, as centre was strongly confounded with many of the potential predictors. We used backwards elimination (p<0.1) to identify the most important predictors, investigating non-linearity using fractional polynomials [17].

Results

Baseline and pharmacokinetic data were available for 71 children from QECH and 56 children from UTH (Table 1). Although median ages were similar (8.4 versus 8.5

**Figure 1** Comparison of nevirapine (NVP) to lamivudine (3TC) and nevirapine to stavudine (d4T) ratios in Triomune® 30 and Triomune® 40 tablets versus ratios of the drugs as recommended for children.

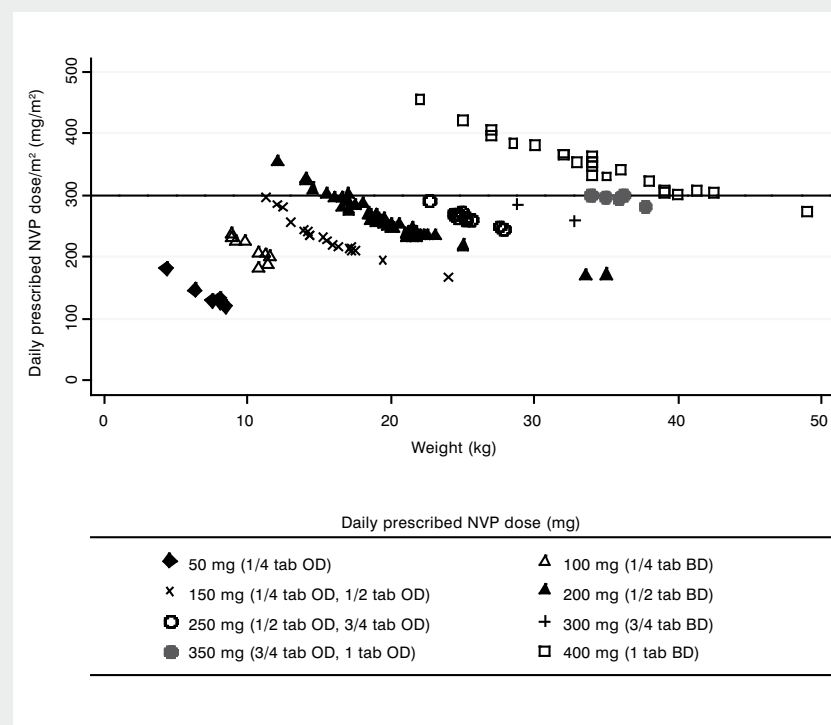


Converting kg to m<sup>2</sup> using standard UK growth charts [11].  
Weights of children in this study are plotted along the top of the graph.

years at QECH and UTH respectively), more young children were enrolled from QECH, with 24% aged 0.5-3 years compared to none from UTH. Children from QECH were on average more malnourished by all anthropometric measures (median (IQR) height-for-age -3.15 (-3.82, -2.42) versus -1.84 (-2.98, -1.17) at UTH; BMI-for-age -0.89 (-1.64, 0.07) versus -0.50 (-1.63, 0.27) respectively). Children at UTH had been on ART for on average 3 months longer (median 8.7 (IQR 5.5, 17) [range 1.4, 46] months) compared to those at QECH (5.9 (3.2, 8.1) [2.2, 38] months).

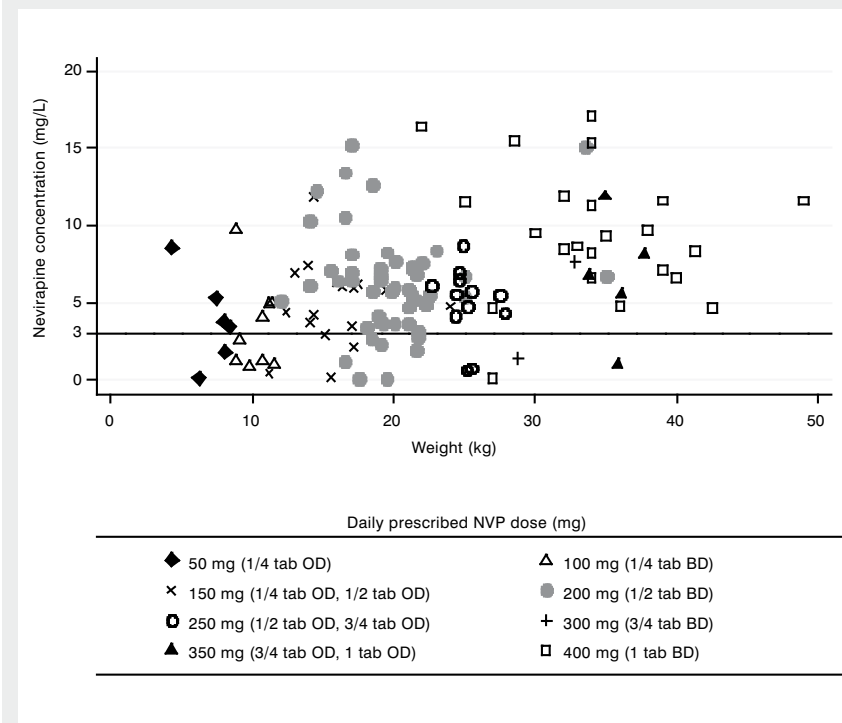
The recommended daily nevirapine dose in children is 300-400 mg/m<sup>2</sup> [9, 18]. Of note, achieving a daily dose of 300 mg/m<sup>2</sup> requires a much higher ratio of nevirapine to

**Figure 2** Nevirapine (NVP) dose/m<sup>2</sup> and concentration by weight.  
(a) Total daily prescribed nevirapine dose/m<sup>2</sup> (mg/m<sup>2</sup>).



Recommended nevirapine dose 300-400 mg/m<sup>2</sup> daily.  
Quarter tablet = 50 mg nevirapine; half tablet = 100 mg nevirapine; whole tablet = 200 mg nevirapine.  
OD = once a day; BD = twice a day.

lamivudine and nevirapine to stavudine for the lightest children than the ratio of these drugs in adult Triomune® 30 or Triomune® 40 tablets. This is illustrated in figure 1, and the weights of children in the study are shown at the top of this figure. All but four of the 27 children weighing <15kg were from QECH; for a fixed dose of lamivudine, children under 15kg have recommended nevirapine doses over 25% greater than that in Triomune®. Uneven dosing and provision of one-quarter and three-quarter tablets occurred at QECH, but not at UTH, where only half or whole Triomune® tablets taken twice daily were used. The median nevirapine dose received was lower at QECH than at UTH (median (IQR) dose/m<sup>2</sup>/day 243 (217, 270) mg/m<sup>2</sup>/day at QECH versus 288 (250, 344) mg/m<sup>2</sup>/day at UTH). Of note, age and nevirapine dose/m<sup>2</sup>/day were strongly

**Figure 2** (b) Nevirapine concentration (mg/L).

Two patients with nevirapine concentrations >20 mg/L excluded.

Therapeutic nevirapine concentration  $\geq 3.0$  mg/L.

Quarter tablet = 50 mg nevirapine; half tablet = 100 mg nevirapine; whole tablet = 200 mg nevirapine.  
OD = once a day; BD = twice a day.

**Table 2** Nevirapine dose and concentration by age group.

	Age group (years)			
	0.5-3 (n=17)	4-6 (n=26)	7-10 (n=41)	11+ (n=43)
<b>Number of Triomune® tablets prescribed</b>				
Quarter once daily (50 mg)	6 (35%)	0	0	0
Quarter twice daily (100 mg)	8 (47%)	0	1 (2%)	0
Quarter once daily + half once daily (150 mg)	3 (18%)	8 (31%)	6 (15%)	1 (2%)
Half twice daily (200 mg)	0	18 (69%)	26 (63%)	8 (19%)
Half once daily + three-quarters once daily (250 mg)	0	0	5 (12%)	7 (16%)
Three-quarters twice daily (300 mg)	0	0	0	2 (5%)
Three-quarters once daily + whole once daily (350 mg)	0	0	1 (2%)	4 (9%)
Whole twice daily (400 mg)	0	0	2 (5%)	21 (49%)
<b>Total daily prescribed nevirapine dose</b>				
mg	100 (50,100)	200 (150,200)	200 (200,200)	350 (250,400)
mg/kg	9.3 (7.9,11.4)	11.1 (10.3,12.5)	9.9 (9.3,10.5)	10.2 (9.4,11.8)
mg/m <sup>2</sup>	204 (147,232)	271 (242,297)	247 (234,263)	298 (258,347)
<b>Nevirapine concentration, mg/L<sup>‡</sup></b>	3.9 (1.5,5.1)	6.4 (4.2,8.1)	5.7 (3.2,7.1)	6.8 (4.7,9.8)
<b>Subtherapeutic nevirapine concentration &lt; 3.0 mg/L</b>	6 (38%) <sup>‡</sup>	3 (12%)	10 (25%) <sup>‡</sup>	4 (9%)

Values given are median (IQR) for continuous variables and number (%) for categorical variables.

<sup>‡</sup> Excluding two patients with nevirapine concentrations >20 mg/L.

confounded, as the youngest children receiving the lowest doses of quarter and half tablets also had the lowest doses in mg/m<sup>2</sup>/day (Figure 2a). Lamivudine and stavudine were closer to the recommended 8 mg/kg/day and 2 mg/kg/day respectively (Table 1), although children at QECH, who were on average younger and received Triomune® 40, tended to have lower lamivudine doses and higher stavudine doses.

Samples were taken later post-dose at QECH (median (IQR) 8.9 (8.3, 9.6) hours versus 3.5 (2.5, 4.2) hours at UTH; Table 1). Two children (one at QECH and one at UTH) had nevirapine concentrations estimated at over 20 mg/L (22 and 31 mg/L

respectively) and were excluded from subsequent analyses, since these high concentrations did not seem to be explained by the stated prescribed dose. Other characteristics of these two children were comparable to the cohort (age 3 and 7 years, BMI-for-age +1.00 and +0.61, height-for-age -3.70 and -2.53, daily dose/m<sup>2</sup> 285 and 253 mg/m<sup>2</sup>/day respectively). Neither child was taking fluconazole prophylaxis or therapy, which is known to increase nevirapine exposure [19].

Of the remaining 125 children, the median (IQR) nevirapine concentrations were 4.8 (2.8, 6.5) and 7.0 (5.4, 10.5) mg/L at QECH and UTH respectively. Nineteen (27%)



of the QECH children and 4 (7%) of the UTH children had subtherapeutic nevirapine concentrations (defined as <3.0 mg/L; median age 8.1 years). Five of these had concentrations below 0.2 mg/L and 11 below 1 mg/L. Thirty-two (26%) children had concentrations over 8 mg/L and 20 (16%) had concentrations over 10 mg/L.

The majority (100 (87%) of the 125) of children were either on no other medications or cotrimoxazole alone. None of the concomitant medications used are known to interact with nevirapine, except isoniazid, which could inhibit the liver enzyme CYP3A4 [20] and potentially increase nevirapine concentrations. However, the three children on isoniazid (all at QECH) all had nevirapine concentrations below the 75<sup>th</sup> centile (2.3, 5.6 and 8.1 mg/L).

Only 2 (7%) of the 28 children prescribed  $\geq 350$  mg/day nevirapine (three-quarter/whole Triumune® tablets; median dose/m<sup>2</sup> 337 (range 274, 454) mg/m<sup>2</sup>) had subtherapeutic nevirapine concentrations compared with 10 (15%) of the 65 children prescribed 200-<350 mg/day nevirapine (half/three-quarter tablets; dose/m<sup>2</sup> 260 (170, 353) mg/m<sup>2</sup>) and 11 (34%) of the 32 children prescribed 50-<200 mg/day (quarter/half tablets; dose/m<sup>2</sup> 214 (120, 297) mg/m<sup>2</sup>) (Figure 2b). Only 1 (3%) of the 30 children prescribed a nevirapine dose adjusted for body surface area of  $\geq 300$  mg/m<sup>2</sup>/day had a subtherapeutic concentration compared with 22 (23%) of the 95 children prescribed <300 mg/m<sup>2</sup>/day.

Age and nevirapine dose received were strongly confounded, as expected (Table 2). However, because younger children have a higher surface area relative to weight, this also led to strong confounding with dose adjusted for surface area: the youngest children received the lowest doses and also received the lowest doses adjusted for surface area compared with the recommended 300-400 mg/m<sup>2</sup>/day [9]. Reflecting underlying variation in surface area adjusted dosing, 38% (6 of 16) in the youngest age group (0.5-3 years old) had subtherapeutic concentrations compared with 12% (3 of 26), 25% (10 of 40) and 9% (4 of 43) in the age groups 4-6, 7-10 and 11+ years respectively.

Children with higher dose/m<sup>2</sup>, higher height-for-age but lower BMI-for-age had higher nevirapine concentrations in univariable and multivariable models (Table 3a). A 50 mg/m<sup>2</sup> higher daily dose of nevirapine was independently associated with a

**Table 3** Predictors of nevirapine concentration. (a) Absolute nevirapine concentration (mg/L).

Factor	Univariable models			Full multivariable model			Final multivariable model*		
	Estimated coefficient	95% CI	P	Estimated coefficient	95% CI	P	Estimated coefficient	95% CI	P
Height-for-age z-score	0.41	0.04,0.78	0.03	0.45	0.06,0.84	0.02	0.37	-0.003,0.74	0.05
BMI-for-age z-score	-0.36	-0.75,0.05	0.08	-0.32	-0.75,0.12	0.2	-0.42	-0.80,-0.04	0.03
Age, years	0.21	0.07,0.34	0.004	0.05	-0.14,0.24	0.6			
Sex, female vs. male	0.31	-0.87,1.49	0.6	0.001	-1.18,1.18	1.0			
Time between dose and sampling, hours	0.04	-0.29,0.37	0.8	0.06	-0.26,0.37	0.7			
Daily dose per 50 mg/m <sup>2</sup> , mg/m <sup>2</sup>	0.96	0.41,1.51	0.001	1.08	0.34,1.81	0.004	0.89	0.32,1.46	0.002

(b) Achieving therapeutic nevirapine concentration ( $\geq 3.0$  mg/L).

Factor	Univariable models			Full multivariable model			Final multivariable model*		
	Estimated odds ratio	95% CI	P	Estimated odds ratio	95% CI	P	Estimated odds ratio	95% CI	P
Height-for-age z score	1.48	1.07,2.06	0.02	1.54	1.04,2.27	0.03	1.48	1.07,2.06	0.02
BMI-for-age z score	0.90	0.64,1.27	0.5	0.96	0.66,1.40	0.8			
Age, years	1.10	0.98,1.23	0.1	1.13	0.95,1.34	0.2			
Sex, female vs. male	0.92	0.35,2.43	0.9	0.85	0.28,2.53	0.8			
Time between dose and sampling, hours	1.02	0.76,1.38	0.9	0.98	0.67,1.45	0.9			
Daily dose per 50 mg/m <sup>2</sup> , mg/m <sup>2</sup>	1.14	0.71,1.82	0.6	0.75	0.36,1.55	0.4			

Two patients with nevirapine concentrations >20 mg/L excluded from all analyses. \* Based on backwards elimination (p<0.1).



0.89 [0.32, 1.46] mg/L higher nevirapine concentration ( $p=0.002$ ). A one unit higher height-for-age was independently associated with a 0.37 (95% CI [-0.003, 0.74]) mg/L higher nevirapine concentration ( $p=0.05$ ), but a one unit lower BMI-for-age was independently associated with a 0.42 [0.04, 0.80] mg/L higher nevirapine concentration ( $p=0.03$ ). There was no evidence that the effect of any of these variables was non-linear. Of note, whilst age and dose/m<sup>2</sup> were both significant univariable predictors, age was a confounder of dose/m<sup>2</sup>, and only dose/m<sup>2</sup> had an independent effect on nevirapine concentration. There was no evidence of an effect of sex on nevirapine concentration (full multivariable model,  $p=1.0$ ).

We examined the above variables with respect to the likelihood of achieving nevirapine concentrations above the therapeutic threshold. A one unit increase in height-for-age was independently associated with a 48 [7, 106] % increase in the odds of achieving a therapeutic nevirapine concentration ( $p=0.02$ , Table 3b). There was no evidence of an association between achieving a therapeutic nevirapine concentration and BMI-for-age, nor dose/m<sup>2</sup> (univariable OR=0.90 [0.64, 1.27],  $p=0.6$ ; 1.14 [0.71, 1.82],  $p=0.6$  respectively), but power was low and height-for-age and dose/m<sup>2</sup> were confounded, with 70% of those with height-for-age z-score<-4 prescribed <250 mg/m<sup>2</sup> daily, compared to just 35% of those with height-for-age z-score $\geq$  -4.

## Discussion

In the absence of appropriate paediatric antiretroviral drug formulations in resource-limited settings, divided adult fixed dose combination (FDC) antiretroviral tablets are often prescribed for use in children. In addition to limited information on the uniformity of distribution of the component drugs in the tablet and difficulties in cutting the unscored tablets accurately, the drugs are in a fixed dose ratio that is not appropriate for children. For example, national guidelines drawn up in 2003 for the delivery of ART to HIV-infected children in Malawi needed to reconcile dosing recommendations for three drugs: two with dosing recommendations based on weight, the third on either surface area or on weight with a step down in dose at age 8 years, reflecting the fact that nevirapine is metabolised more rapidly in younger children. The guidelines needed to be applied in District units (or smaller health

centres) where staff training and facilities would be extremely limited. Provision of individual syrups and dosing by surface area were considered impossible on a national basis. Triomune® 40 (but not 30) was already in use and about to be recommended for adults in the national programme, but it was clearly not possible to dose all 3 components according to individual recommendations using Triomune® 40 for children. Given a choice between separate paediatric formulations, underdosing nevirapine (particularly in younger children) or overdosing lamivudine and stavudine, a pragmatic decision was made to base Triomune® 40 recommendations on doses for lamivudine and stavudine, i.e. on a mg/kg basis, with a linear relationship between weight and dose, and accepting that nevirapine was likely to be underdosed in the youngest children.

Our results indicate that using divided Triomune® tablets in this way can lead to subtherapeutic nevirapine concentrations at any age and body weight when these are associated with low nevirapine doses/m<sup>2</sup>/day. Younger, smaller children who are more likely to receive quarter tablets and have higher surface area for weight are at particular risk, with nearly half of those receiving quarter tablets either once or twice daily having subtherapeutic concentrations. Because of the higher viral loads and faster metabolism in young children, the potential for development of drug resistant virus is significant [21, 22]. Therefore quarter Triomune® tablets should be avoided where appropriate alternative ART formulations exist. We did not measure lamivudine or stavudine levels in plasma; although this is not the active moiety of these drugs, which act intracellularly, the doses prescribed suggest that subtherapeutic levels of these drugs could also have occurred in some children. This is of particular concern for lamivudine given the low genetic barrier to development of the M184V mutation, and recent findings that 8 mg/kg/day dosing may also be too low in children under 6 years of age [23].

We found that the strongest predictor of nevirapine level was the dose prescribed in mg/m<sup>2</sup>/day and subtherapeutic concentrations were infrequently observed in those children receiving a nevirapine dose of  $\geq 300$  mg/m<sup>2</sup>/day. Body surface area measurements are difficult to calculate and errors have been reported [24]. A recent paper describing underdosing of many antiretrovirals in children points out non-concordance of dosing when calculated using mg/kg versus mg/m<sup>2</sup> recommendations for nevirapine in particular [4]. In collaboration with leading HIV

paediatric pharmacologists, WHO has recently developed standardised weight-band based dosing tables for all antiretrovirals used in children, using surface area dose calculations as the basis for conversion into weight bands. This should simplify dosing of antiretrovirals in resource-limited settings [9]. Of note, over a quarter of the children in our study had plasma nevirapine concentrations over 8 mg/L and 16% had concentrations over 10 mg/L, although overt toxicity was not observed.

Our results suggest that nevirapine concentrations may be reduced in stunted but increased in wasted children, with lower height-for-age (a pseudo-measure for stunting) independently associated with lower nevirapine concentrations and lower BMI-for-age (a pseudo-measure for wasting) independently associated with higher nevirapine concentrations. One possible explanation is that wasted children will be older than well-nourished children of the same weight, and so will metabolise the same dose of nevirapine more slowly. As malnutrition is common in HIV-infected children in sub-Saharan Africa and is often used as a clinical criteria of eligibility to access ART, these results carry important implications for clinical practice. Further pharmacokinetic studies in a larger number of children taking a range of antiretroviral drugs is required to confirm these findings. It is possible that different recommendations for dosing malnourished children may be required, although in general if food is available, weight gain and growth after starting ART is rapid. Doses also need to be monitored carefully with any rapid changes in height and/or weight. However, children in this study remained stunted and wasted 3 or more months after starting ART.

Although samples were on average taken later post-dose at QECH compared to UTH (median 8.9 vs 3.5 hours), the long half-life of nevirapine (median 25.5, range [12.1, 105.2] hours in children [5], similar to median 24.3 hours observed in adults [25]) leads to only a minimal difference between nevirapine peak and trough concentrations at steady state. Indeed we observed no effect of dose timing in the multivariable analysis. In addition, the lower limit of the therapeutic range for nevirapine (3.0 mg/L) is based on a study in which samples were taken at random times [14]. Two children (one from each centre) had nevirapine concentrations estimated at over 20 mg/L and were excluded from the main analysis. These high concentrations did not seem to be explained by the stated prescribed dose, which was comparable to the other children, or by concomitant medications. Further, children had been on nevirapine for median 6.5 months, so gastrointestinal problems

which could affect absorption are unlikely. An alternative explanation is a possible pharmacogenomic or -genetic effect, as the CYP2B6 T/T genotype at position 516 is more common in African-Americans than in European-Americans and is associated with greater efavirenz exposure [26] and, to a lesser extent, with greater nevirapine exposure [27]. Misunderstanding instructions about how many pieces of tablet or tablets to give could also have occurred. A limitation of our study is that we did not measure adherence, nor were any drugs taken under direct observation; nevirapine concentrations may therefore reflect adherence as well as prescribed dose. Those children with very low nevirapine concentrations are unlikely to have taken ART for several days, emphasising the difficulties of administering drugs to young children. In addition, we did not have viral loads to be able to relate nevirapine levels to treatment success or failure.

Nevirapine pharmacokinetics have been studied recently in 34 Thai children (median age 8.4 years) who similarly received divided tablets (GPO-VIR S30®, equivalent to Triomune® 30) [5]. In this study 33 of the 34 children achieved therapeutic concentrations (defined as  $\geq 3.4$  mg/L). However none were receiving quarter tablets and the mean daily nevirapine dose was considerably higher at 328 mg/m<sup>2</sup>, compared to 265 mg/m<sup>2</sup> in our study. A recent comparison of the pharmacokinetics of liquid formulations versus split Triomune® 40 in 18 Malawian children (median age 7 years) concluded that although quartered multiples of FDC tablets are reasonable for use in larger children, liquid formulations are better for smaller children [6].

Our findings demonstrate that it is difficult to achieve adequate nevirapine levels [28] in young children with divided Triomune® tablets without a risk of overdosing with stavudine, especially when using Triomune® 40. Therefore, for young children, paediatricians currently face a difficult decision, as increasing the Triomune® dose to ensure adequate nevirapine will consequently increase the dose of lamivudine and stavudine. Higher doses of stavudine in particular may lead to toxicity; its main side effect, peripheral neuropathy, can be difficult to identify in children and may be irreversible. Use of Triomune® 30 rather than Triomune® 40 would enable higher doses of nevirapine and lamivudine to be given and, as higher doses of lamivudine may anyway be beneficial in younger children, this would be preferable [23]. An alternative strategy would be to supplement quarter Triomune® tablets with

additional nevirapine syrup, although this may not always be available and would be more difficult for caregivers. Of note, despite the low nevirapine levels achieved and the potential for development of resistance, the children in this cohort continue to go to school, put on weight and come to clinic, in contrast to the high mortality observed in the cohort when ART was not available [8]. As a result of these data and also because Triomune 30 is now available in Malawi, national paediatric ART dosing guidelines have been amended: the Triomune® dose has been increased in younger children such that few children now receive less than 300 mg/m<sup>2</sup> nevirapine, while accepting a modest increase in the doses of lamivudine and stavudine.

Cipla Pharmaceuticals are developing FDC antiretroviral tablets in two formulations suitable for infants and children with a higher proportion of nevirapine, Pedimune® Baby (6 mg stavudine, 30 mg lamivudine, 50 mg nevirapine) and Pedimune® Junior (twice Baby dose). Tablets are scored to enable dividing for accurate dosing and are dispersible. They are currently undergoing clinical investigation but are not yet pre-qualified by WHO and therefore so far not accessible to national ART programmes. The availability of these and other paediatric generic FDC tablets for resource-limited settings is eagerly awaited and long overdue. It is a high priority for successful scaling up ART for children.

## Acknowledgements and sources of support

We would like to thank the families and children, and staff from the Queen Elizabeth Central Hospital, Blantyre, Malawi and the University Teaching Hospital and School of Medicine, Lusaka, Zambia. The Canadian Government provided WHO-AFRO-Canadian funds as part of a grant to WHO to support ART scale up in Malawi. The CHAP trial and its follow-up was funded by the Department for International Development, UK.

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A stylized illustration of several hands of different sizes and shades of gray reaching upwards from the bottom of the frame. The background is a dark gray gradient with a pattern of small, light gray circles. The hands are positioned at various heights and angles, creating a sense of movement and reaching towards the top of the page.

## Chapter 3

Pharmacokinetics of two generic fixed-dose combinations for HIV-infected children (Pedimune Baby & Pedimune Junior) are similar to the branded products in healthy adults

Rafaëlla L'homme<sup>1,2</sup>, Tim Dijkema<sup>3</sup>, Adilia Warris<sup>2,4</sup>, Andre van der Ven<sup>2,5</sup>, Diana Gibb<sup>6</sup>, David Burger<sup>1,2</sup>

<sup>1</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands; <sup>3</sup> Clinical Research Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>4</sup> Department of Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>5</sup> Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>6</sup> Medical Research Council, Clinical Trials Unit, London, UK

*Journal of Antimicrobial Chemotherapy* 2007; 59:92-6

## Abstract

**Objectives:** Cipla Pharmaceuticals have developed generic fixed-dose combinations of stavudine, lamivudine and nevirapine for HIV-infected children (Pedimune Baby and Junior). We determined the pharmacokinetic profiles of stavudine, lamivudine and nevirapine in Pedimune and compared these with the branded products.

**Methods:** This phase-I, comparative, single-center, open-label, three-period, single-dose study was designed as a pilot study to exclude large differences in pharmacokinetics. Six healthy males were randomized to the following regimen sequences: ABC; ACB; BCA; BAC; CAB; CBA (A = reference, B = Pedimune Baby, C = Pedimune Junior). Single doses of medication were administered at 3 time points 4 weeks apart. An 8h pharmacokinetic curve was recorded at day 1 of every cycle after medication intake. In addition, blood samples were taken on day 2, 3, 4, 8 and 15.

**Results:** Nonparametric statistical tests revealed no statistically significant differences in  $C_{max}$  ( $0.173 \leq P \leq 0.753$ ) and  $T_{max}$  ( $0.317 \leq P \leq 1.000$ ) of stavudine, lamivudine and nevirapine between the 2 Pedimune formulations and the branded drugs. Also, there were no significant differences in  $AUC_{0-\infty}$  of stavudine, lamivudine and nevirapine between Pedimune Junior and the branded drugs ( $0.345 \leq P \leq 0.600$ ) and between Pedimune Baby and the branded drug for nevirapine ( $P=0.463$ ). In contrast, the  $AUC_{0-\infty}$  of stavudine (mean change: +21%;  $P=0.046$ ) and lamivudine (mean change: +14%;  $P=0.028$ ) differed significantly between Pedimune Baby and the branded drugs, but these changes were considered not clinically significant.

**Conclusions:** The pharmacokinetic profiles of stavudine, lamivudine and nevirapine in Pedimune Baby and Junior are comparable to the branded products. Based on these results, it is acceptable to test the pharmacokinetics and dosing requirements of Pedimune in HIV-infected children.

## Introduction

In well resourced countries, Anti-Retroviral Therapy (ART) with three or more potent drugs has resulted in major reductions in morbidity and mortality of HIV-infected adults and children [1, 2].

There are now intensive efforts by governments and non-governmental organizations to increase the number of people being treated with ART in resource-limited parts of the world where 90% of infected individuals live. For HIV-infected adults in these settings, pharmaceutical companies have reduced drug costs through separate pricing, and generic manufacturers have been allowed to produce ART combinations at lower costs without facing patent claims.

Cipla Pharmaceuticals is an India-based generic manufacturer that has produced a fixed-dose combination (FDC) tablet for HIV-infected adults, (Triomune - 30 or 40mg stavudine, 150mg lamivudine, 200mg nevirapine), to be taken twice-daily without food restriction. Bioequivalence and clinical efficacy have been demonstrated [3-5]. At a price of less than 1 euro/day, Triomune is now a reasonable option for many adult patients, and is frequently used in access-to-care programs.

In 2005, it was estimated that at least 660,000 children were in need of ART [6], of whom 90% live in sub-Saharan Africa. However, fewer than 4% of individuals receiving ART in 2005 were children. There are several reasons for this including difficulties in making an early diagnosis of HIV in HIV-exposed infants and lack of personnel trained in paediatric ART management. However, arguably the biggest barrier is lack of appropriate formulations of ART, including FDC tablets, and simple dosing tables. Paediatric brand liquid formulations are expensive, may require water for reconstitution, which is not always available in good quality, and may require refrigeration, which is a particular problem in rural areas. Further, until recently, there were no FDC tablets for children. Triomune is sometimes prescribed dosed as half or quarter tablets. However, lack of scoring can result in inaccurate breaking and hence inaccurate dosing. Of more concern are preliminary data from a study in Malawian and Zambian children which showed that nevirapine underdosing was common, particularly in the youngest children receiving quarter tablets of Triomune [7].

To address this issue, Cipla Pharmaceuticals have recently developed two generic fixed-dose combinations for HIV-infected children (Pedimune Baby and Pedimune Junior) including the same agents as in Triomune, but in a different dose ratio, with higher dose of nevirapine. Pedimune Baby contains 6mg stavudine, 30mg lamivudine and 50mg nevirapine, while Pedimune Junior contains double the dose. Pedimune tablets are small, dispersible or crushable, and scored.

The primary objective of this pilot study was to determine the pharmacokinetic profile of stavudine, lamivudine and nevirapine in Pedimune Baby and Pedimune Junior after single-dose in healthy males, and to compare this to the individual branded products. The study was conducted in the Netherlands as a prelude to a pharmacokinetic study of Pedimune in HIV-infected children in Zambia which has recently started.

## Materials and methods

This phase-I, comparative, single-center, open-label, three-period, single-dose study was not designed as a bioequivalence study but as a pilot study to exclude large differences in pharmacokinetic profiles. Healthy male subjects aged 18 to 65 years were eligible for enrollment after pre-entry and laboratory evaluation. Subjects who tested positive for HIV and/or hepatitis B or C and subjects with abnormal clinical laboratory test results were excluded. Subjects were not allowed to take any concomitant drug (for two weeks preceding dosing), except for paracetamol and loperamide. The study protocol was reviewed and approved by the Ethics Committee of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Informed consent was obtained from all subjects before enrollment.

Subjects were randomized to one of the following regimen sequences: ABC; ACB; BCA; BAC; CAB; CBA:

*Regimen A (reference):* 24mg stavudine (24mL Zerit powder for suspension 1 mg/mL, Bristol-Myers Squibb), 120mg lamivudine (12mL Epivir solution 10mg/mL, GlaxoSmithKline) and 200mg nevirapine (one Viramune tablet of 200mg, Boehringer Ingelheim).

*Regimen B (test 1):* 4 combined-formulation tablets consisting of 6mg stavudine, 30mg lamivudine, and 50mg nevirapine (Pedimune Baby, Cipla Pharmaceuticals).

*Regimen C (test 2):* 2 combined-formulation tablets consisting of 12mg stavudine, 60mg lamivudine, and 100mg nevirapine (Pedimune Junior, Cipla Pharmaceuticals).

Single doses of medication, normalized to 200mg of nevirapine, were administered at 3 time points 4 weeks apart. Medication was taken orally after a minimum fast of 3 hours. One of the investigators directly observed medication ingestion. Breakfast and lunch were standardised on the day of medication ingestion and were administered 2 and 5 hours after intake, respectively.

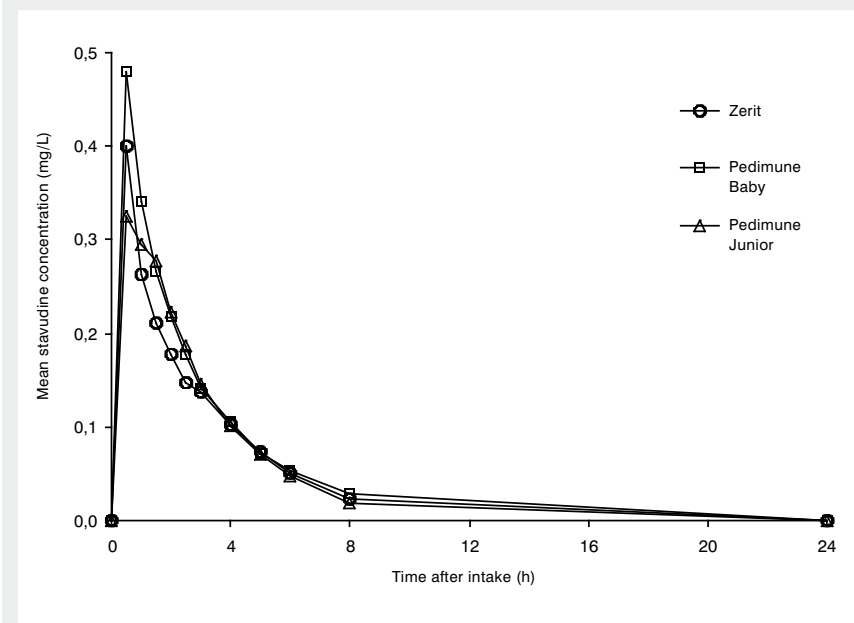
Blood was collected just before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 24, 48, 72, 168 and 336 hours after intake. Blood samples were stored at 2-8°C for maximum 8 hours until being centrifuged. Immediately after centrifugation, plasma was separated and stored at -40°C until analysis. Plasma concentrations of stavudine and lamivudine were determined in all samples up to 24 hours after intake by a validated HPLC assay with ultraviolet (UV) detection [8]. Plasma concentrations of nevirapine were determined in all samples by a validated HPLC assay with UV detection, modified from a method published by Hollanders et al [9]. The lower and upper limits of quantification for the modified assay were 0.03 and 15 mg/L, respectively. The intraday precision ranged from 0.4% to 11.4%, while additional variation as a result of performing the assay on different days ranged from 0.0% to 2.1%. The accuracy of the assay ranged from 100.1% to 104.8%.

The pharmacokinetic (PK) parameters  $C_{max}$  and  $T_{max}$  were calculated directly from observations of plasma concentrations. Non compartmental pharmacokinetic analysis was performed by using WinNonlin software package (version 4.1; Pharsight Corporation, Mountain View, Calif.) to determine the PK parameter  $AUC_{0-\infty}$ .

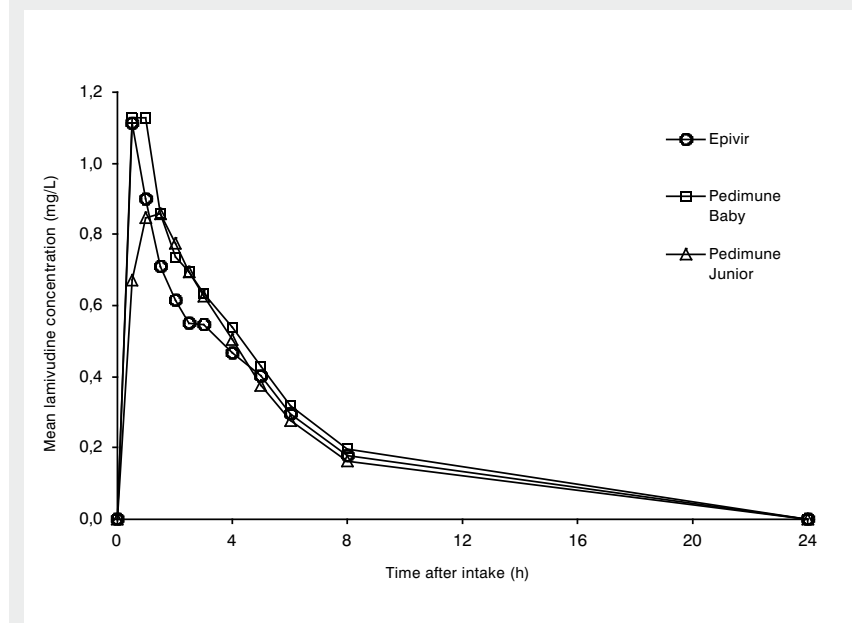
Wilcoxon's signed rank test was used to compare  $C_{max}$ ,  $AUC_{0-\infty}$  and  $T_{max}$  between the generic and branded formulations. Descriptive statistics were carried out by using SPSS, software version 12.0 [SPSS Inc., 1989-2003].



**Figure 1** Mean stavudine concentrations (mg/L) in 6 healthy males after intake of a single dose of Zerit, Pedimune Baby and Pedimune Junior.



**Figure 2** Mean lamivudine concentrations (mg/L) in 6 healthy males after intake of a single dose of Epivir, Pedimune Baby and Pedimune Junior.



## Results

Six healthy white male subjects were enrolled in the protocol. The median age, height, and body weight (range) were 43 (21-63) years, 1.84 (1.74-1.98) m, and 86.5 (69.0-100.0) kg, respectively. All 6 males completed the study.

Plasma concentrations of stavudine, lamivudine and nevirapine after single doses of the branded drugs (A), Pedimune Baby (B) and Pedimune Junior (C) are illustrated in Figures 1, 2 and 3, respectively. There are no large differences between the different plasma concentration-time curves.

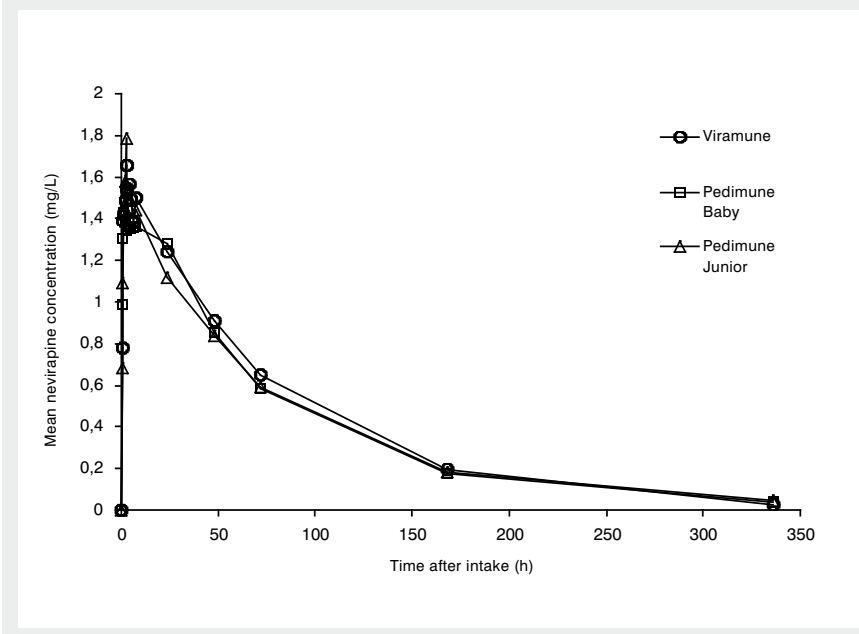
Primary pharmacokinetic (PK) parameters of stavudine, lamivudine and nevirapine after single doses of the branded drugs (A), Pedimune Baby (B) and Pedimune Junior (C) are shown in Table 1.

Nonparametric statistical tests revealed no statistically significant differences in the  $C_{max}$  ( $0.173 \leq P \leq 0.753$ ) and  $T_{max}$  ( $0.317 \leq P \leq 1.000$ ) of stavudine, lamivudine and nevirapine between the two Pedimune formulations and the branded drugs. Furthermore, no statistically significant differences in the  $AUC_{0-\infty}$  of stavudine, lamivudine and nevirapine were found between Pedimune Junior and the branded drugs ( $0.345 \leq P \leq 0.600$ ). The  $AUC_{0-\infty}$  of stavudine ( $P = 0.046$ ) and lamivudine ( $P = 0.028$ ) differed significantly between Pedimune Baby and the branded formulations, while there was no significant difference in the  $AUC_{0-\infty}$  of nevirapine ( $P = 0.463$ ). For Pedimune Baby the mean  $AUC_{0-\infty}$  of stavudine and lamivudine was 21% and 14% higher compared to the branded drugs, respectively.

Treatments were generally well tolerated. No adverse events were reported after intake of Pedimune Junior. Reported mild adverse events, possibly related to treatment, were diarrhoea (1 out of 6 subjects on branded products and 1 out of 6



**Figure 3** Mean nevirapine concentrations (mg/L) in 6 healthy males after intake of a single dose of Viramune, Pedimune Baby and Pedimune Junior.



subjects on Pedimune Baby) and nausea (2 out of 6 subjects on branded products). All adverse events resolved spontaneously.

## Discussion

The present pilot study shows that the pharmacokinetic profiles of stavudine, lamivudine and nevirapine in Pedimune Baby and Junior are comparable to the individual branded products after intake of single doses.

A bioequivalence study is typically conducted as a single-dose, crossover trial. With three different formulations a minimum number of 6 subjects was needed for this pilot study to test all possible sequences. The study was not powered to prove bioequivalence, but to exclude large differences (> 50%) in pharmacokinetic parameters.

**Table 1** Pharmacokinetic parameters of stavudine, lamivudine and nevirapine in 6 healthy males after intake of a single dose of branded drugs (Zerit, Epivir, Viramune), Pedimune Baby and Pedimune Junior.

PK parameters	Branded drugs (A)	Pedimune Baby (B)	Pedimune Junior (C)
<b>Stavudine</b>			
$C_{max}$ (mg/L)	0.43 (0.19-0.54)	0.49 (0.26-0.77)	0.38 (0.29-0.52)
$AUC_{0-\infty}$ (mg-h/L)	1.05 (0.73-1.54)	1.27 (0.85-1.71)	1.08 (0.67-1.51)
$T_{max}$ (h)	1.08 (0.50-4.00)	0.58 (0.50-1.00)	0.83 (0.50-1.50)
<b>Lamivudine</b>			
$C_{max}$ (mg/L)	1.20 (0.55-1.56)	1.33 (0.70-1.92)	1.04 (0.83-1.24)
$AUC_{0-\infty}$ (mg-h/L)	4.48 (3.48-5.59)	5.09 (3.57-6.50)	4.41 (3.66-5.75)
$T_{max}$ (h)	1.25 (0.50-5.00)	1.00 (0.50-2.50)	1.67 (0.50-4.00)
<b>Nevirapine</b>			
$C_{max}$ (mg/L)	1.96 (1.64-2.29)	1.71 (0.96-2.91)	1.93 (1.48-2.48)
$AUC_{0-\infty}$ (mg-h/L)	136.05 (85.04-203.58)	127.92 (88.15-199.39)	127.53 (77.38-246.85)
$T_{max}$ (h)	2.33 (1.00-5.00)	6.25 (1.00-24.00)	2.42 (1.00-5.00)

Values given are mean (range).

Although the formulations are designed for HIV-infected children, it is generally not accepted to study pharmacokinetics of new agents or new formulations in healthy children. Therefore, healthy adults were included. Due to a possible effect of endogenous or exogenous estrogenic hormones on the pharmacokinetics of nevirapine, the effect of nevirapine on oral contraceptives, more toxicity of nevirapine in healthy females, and the small sample size, we only included male subjects.

Nonparametric statistical tests revealed no statistically significant differences in the  $C_{max}$  and  $T_{max}$  of stavudine, lamivudine and nevirapine between the two Pedimune formulations and the branded drugs. Moreover, no statistically significant differences were found for the  $AUC_{0-\infty}$  of stavudine, lamivudine and nevirapine between Pedimune Junior and the branded drugs. While there was no significant difference in the  $AUC_{0-\infty}$  of nevirapine between Pedimune Baby and Viramune, the  $AUC_{0-\infty}$  of stavudine and lamivudine was significantly higher (21 and 14%, respectively) after

intake of Pedimune Baby when compared to the individual branded formulations. However, we do not consider these differences in  $AUC_{0-\infty}$  (far below 50%) to be large and find the pharmacokinetic profiles of stavudine, lamivudine and nevirapine in Pedimune Baby and Junior comparable to the individual branded products. In addition, it would be more worrisome when  $AUC_{0-\infty}$  of stavudine and/or lamivudine were lower instead of higher, as observed here. In general, virological failure is much more difficult to manage than toxicity.

Cipla Pharmaceuticals is currently conducting a formal bioequivalence study on Pedimune prior to applications for registration, and to meet prequalification criteria set by the WHO. Prior to results being available from this, we believe that the information from this independent pilot study showing comparable pharmacokinetic profiles of the three agents (stavudine, lamivudine and nevirapine) between the newly-developed Pedimune tablets and the branded products, is sufficient for us to start a larger pharmacokinetic study in African HIV-infected children in Zambia who are a key target population for use of Pedimune. The children in our trial are closely monitored for potential toxicity and virological failure, because bioequivalence of the Pedimune tablets still needs to be proven. Given that large numbers of children are either receiving no Anti-Retroviral Therapy (ART), or inappropriate ART doses from part Triomune tablets, it is imperative that fixed-dose combinations of appropriate formulations and doses are tested urgently and then become available and licensed for children as soon as possible.

In conclusion, the pharmacokinetic profiles of stavudine, lamivudine and nevirapine in Pedimune Baby and Junior are comparable to the individual branded products. Based on the results of this pilot study, it is acceptable to start testing the pharmacokinetics and dosing requirements of Pedimune Baby and Junior in HIV-infected children, while monitoring closely for potential toxicity and virological failure.

## Acknowledgements

We would like to thank the laboratory technicians of the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, for the analysis of the samples.

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## Chapter 4

**Nevirapine, stavudine and  
lamivudine pharmacokinetics  
in African children  
on paediatric fixed-dose  
combination tablets**

Rafaëlla L'homme<sup>1,2</sup>, Desiré Kabamba<sup>3</sup>, Fiona Ewings<sup>4</sup>, Veronica Mulenga<sup>3</sup>, Chipepo Kankasa<sup>3</sup>, Margaret Thomason<sup>4</sup>, Sarah Walker<sup>4</sup>, Chifumbe Chintu<sup>3</sup>, David Burger<sup>1,2</sup>, Diana Gibb<sup>4</sup>

<sup>1</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands; <sup>3</sup> Department of Paediatrics and Child Health, University Teaching Hospital, Lusaka, Zambia; <sup>4</sup> Medical Research Council, Clinical Trials Unit, London, UK

*AIDS 2008; 22:557-65 (Fast Track)*

## Abstract

**Objective:** Triomune Baby and Junior have been developed in response to the urgent need for appropriate paediatric fixed-dose combination antiretroviral tablets, with higher nevirapine to stavudine and lamivudine ratios than adult tablets in accordance with paediatric recommendations. We determined whether this ratio results in optimal exposure in the target population.

**Methods:** Seventy-one Zambian children were treated with Triomune Baby or Junior dosed according to weight-bands. After 4 weeks or more, a 12-hour pharmacokinetic curve was recorded. Antiretroviral plasma concentrations were assayed by high-performance liquid chromatography.

**Results:** Six children were excluded because of poor adherence. Of the remaining 65, 24 (37%) were female, 24 (37%) weighed less than 15kg and most were malnourished. Mean (range) nevirapine  $C_{12h}$ ,  $C_{max}$  and  $AUC_{12h}$  of 6.0 (1.4,16.9) mg/L, 10.0 (3.8,22.5) mg/L and 94.4 (32.1,232) h.mg/L were higher than those reported in adults. Nevirapine  $C_{12h}$  was subtherapeutic ( $<3.0$  mg/L) in four (6%) children. Mean stavudine and lamivudine  $C_{12h}$ ,  $C_{max}$ ,  $AUC_{12h}$  ( $<0.015$  mg/L, 0.45 mg/L, 1.05 h.mg/L and 0.09 mg/L, 1.33 mg/L, 5.42 h.mg/L) were comparable to adults. There was no evidence of a difference in nevirapine  $AUC_{12h}$  across weight-bands ( $p=0.2$ ), while the difference in stavudine ( $p=0.0003$ ) and lamivudine  $AUC_{12h}$  ( $p=0.01$ ) was driven by the single weight-band with unequal dosing.

**Conclusions:** Nevirapine concentrations were higher but more variable than those in adults; pharmacokinetic parameters of stavudine and lamivudine were comparable to adults. As nevirapine underdosing is of greater concern than overdosing, the Triomune Baby and Junior ratio appears to be appropriate for children weighing 6kg and over. Further research is required for children under 6kg.

## Introduction

Of 2.3 million children living with HIV in 2006, almost 90% in sub-Saharan Africa, an estimated 780,000 urgently require antiretroviral (ARV) therapy [1]. Where liquids are available, they are costly, have short shelf-lives, and are difficult to transport and store [2]. Due to lack of availability of affordable and appropriate paediatric ARV drug formulations, divided adult fixed-dose combination tablets (FDCs) are frequently provided for children. However, adult FDCs do not allow easy dose adjustment as a child grows. Further, and more importantly, drug ratios in these adult FDCs are not correct for children. Our previous study in 127 HIV-infected Malawian and Zambian children receiving divided adult FDCs (Triomune; Cipla Pharmaceuticals, India; 200mg nevirapine, 30 or 40mg stavudine, 150mg lamivudine) demonstrated a risk of nevirapine underdosing [3], due to children, particularly the youngest, metabolizing nevirapine more rapidly than adults. Underdosing is a major threat to long-term success of ARV therapy, as lack of potency in suppressing viral replication will result in development of mutations, particularly against drugs with a low genetic barrier to resistance such as nevirapine and lamivudine, and limit subsequent treatment options [4, 5]. This is of particular concern for children facing a life-time requirement of ARV therapy.

To address this problem, Cipla Pharmaceuticals have developed small, dispersible, crushable, scored FDCs for HIV-infected children in two sizes (Triomune Baby and Junior) with relatively higher nevirapine versus stavudine and lamivudine dose ratios compared to the adult FDC, in accordance with paediatric dosing recommendations [6]. In our independent pilot bioequivalence study, the pharmacokinetics (PK) of Triomune Baby and Junior were similar to branded products in six healthy males [7]. Formal bioequivalence was demonstrated by Cipla Pharmaceuticals (data on file).

Here we studied the PK of nevirapine, stavudine and lamivudine in Zambian HIV-infected children prescribed Triomune Baby or Junior twice daily according to surface-area derived weight-bands, to determine whether the dose ratio results in optimal ARV exposure in the target population.

## Methods

### Study population and design

CHAPAS1 is an open, randomised, controlled, phase I/II trial designed to assess the appropriate dosing of, and adherence to, Triomune Baby (50mg nevirapine, 6mg stavudine, 30mg lamivudine) and Junior (double Baby dose). Two hundred HIV-infected children aged 3 months to 14 years weighing <30kg who fulfill WHO criteria for initiating ARV therapy have been enrolled at the University Teaching Hospital, Lusaka, Zambia. Children were randomised in a 1:1 ratio to take Triomune Baby or Junior either immediately at full dose in a twice-daily schedule, or in a once-daily dose escalation schedule with an additional stavudine/lamivudine tablet (Lamivir-S, Cipla Pharmaceuticals) for the first 14 days, followed by full dose. Children previously treated with ARVs, including for prevention of mother-to-child HIV transmission, were excluded. Further exclusion criteria were severe laboratory abnormalities, active opportunistic infection, and treatment with any medication known to be contra-indicated in combination with nevirapine, stavudine and/or lamivudine.

The first 64 children enrolled in CHAPAS1 were to participate in the PK substudy, with 16 per age-group <3, 3-6, 7-10 and 11-14 years. These children had to fulfill all enrolment criteria for CHAPAS1 and also not suffer from illnesses that could influence the PK of the ARVs such as diarrhea, vomiting, renal or liver disease, and not be taking concomitant medication with interactions to the ARVs.

Full nevirapine dosing was chosen to aim for daily doses of 300-400 mg/m<sup>2</sup> [6] using estimated body surface area (BSA) for weight converted into weight-bands (3-<6, 6-<10, 10-<15, 15-<20, 20-<25, 25-<30kg) [8]. Daily stavudine and lamivudine doses were targeted to 2 and 8 mg/kg respectively. Nevirapine dose ranges in mg/m<sup>2</sup> and stavudine and lamivudine dose ranges in mg/kg (Table 1) are the result of the same dose in mg being given over weight (and BSA) ranges. Of note, one weight-band (15-<20kg) has unequal morning and evening doses.

The protocol was reviewed and approved by the Ethics Committees of the University of Zambia, Lusaka and University College London. Written informed consent was obtained from parents or guardians, and children where appropriate.

**Table 1** Dosing schedule by weight-band and associated nevirapine (NVP), stavudine (d4T) and lamivudine (3TC) doses.

Weight-band, kg	Type of tablets	Number of tablets daily		Daily dose, mg			Daily dose, mg/m <sup>2</sup> * & **		Daily dose, mg/kg *	
		a.m.	p.m.	NVP	d4T	3TC	NVP		d4T	3TC
3-<6	Triomune Baby (NVP 50mg, d4T 6mg, 3TC 30mg)***	1	1	100	12	60	294-476		2.0-4.0	10.0-20.0
6-<10		1½	1½	150	18	90	306-441		1.8-3.0	9.0-15.0
10-<15	Triomune Junior (NVP 100mg, d4T 12mg, 3TC 60mg)***	1	1	200	24	120	308-408		1.6-2.4	8.0-12.0
15-<20		1	1½	250	30	150	316-385		1.5-2.0	7.5-10.0
20-<25		1½	1½	300	36	180	326-380		1.4-1.8	7.2-9.0
25-<30		2	2	400	48	240	364-435		1.6-1.9	8.0-9.6

\* Most recent WHO daily dose recommendations are 300-400 mg/m<sup>2</sup> nevirapine, 2 mg/kg stavudine and 8 mg/kg lamivudine [6].

\*\* Based on estimated equivalent body surface area for weight [8].

\*\*\* Adult Triomune 30 or 40 tablets contain 200mg nevirapine, 30 or 40mg stavudine, 150mg lamivudine.

### Blood collection and drug concentration assays

At least four weeks after starting Triomune Baby or Junior, a 12-hour PK curve was undertaken, following directly observed medication ingestion. Non-breast-fed children fasted for ≥ 3 hours before intake, and standardized meals were given 1-2, 4-6 and 8-12 hours after intake. Two mL of blood was collected just before and 1, 2, 4, 6, 8 and 12 hours after intake. Plasma was separated and stored at -80°C until transportation to the Netherlands on dry-ice.

Plasma concentrations of nevirapine, stavudine and lamivudine were assayed at the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, using two validated HPLC assays with ultraviolet detection [9, 10]. The plasma drug concentration 12 hours post-dose (C<sub>12h</sub>) and maximum concentration (C<sub>max</sub>) were determined directly from concentration-time data. The area under the concentration-time curve 0-12 hours post dose (AUC<sub>12h</sub>) was evaluated using the linear-log trapezoidal rule.

Children were excluded if there was evidence of non-adherence, i.e. if the ratio between nevirapine C<sub>12h</sub> and predose concentration was more than 2 and

non-adherence was independently suspected by the study team, or if this ratio was more than 4 without suspected non-adherence.

### Statistical methods

Weight-for-age, height-for-age and body mass index (BMI)-for-age z-scores were calculated using the 1990 British Growth Charts [11] in Stata 9 [12] and CD4-for-age using published age-related reference ranges [13]. Nevirapine, stavudine and lamivudine AUC<sub>12h</sub> were compared across weight-bands and age groups using analysis of variance adjusted for randomisation.

## Results

Of the 71 children enrolled in the PK substudy, six were excluded due to evidence of non-adherence. These six, aged 10 months to 12 years, had similar weights and CD4% to the rest of the cohort, and daily prescribed nevirapine doses of 363-424 mg/m<sup>2</sup>. Of the remaining 65 children, 16, 18, 16 and 15 were aged <3, 3-6, 7-10 and 11-14 years respectively. The majority were malnourished and moderately to severely immunodeficient (Table 2). The mean (range) daily prescribed nevirapine dose at enrolment was 370 (317, 486) mg/m<sup>2</sup>. Of note, two children who were dosed at enrolment according to screening weight (10 and 11kg) had experienced weight loss (both to 9.8kg) by the time of enrolment, and therefore received doses equating to 427 and 416 mg/m<sup>2</sup> respectively at enrolment. Thirty-one (48%) and 34 (52%) were randomised to the full dose and dose escalation arms of CHAPAS1 respectively.

The median (range) time from ARV therapy initiation to PK day was 27 (26, 56) days. Seven children gained and one lost weight after enrolment such that they were in the next higher (three 6-<10kg, one 10-<15kg, two 15-<20kg and one 20-<25kg at enrolment) or next lower (one 15-<20kg) weight-band on PK day. Doses were adjusted after the end of the PK day.

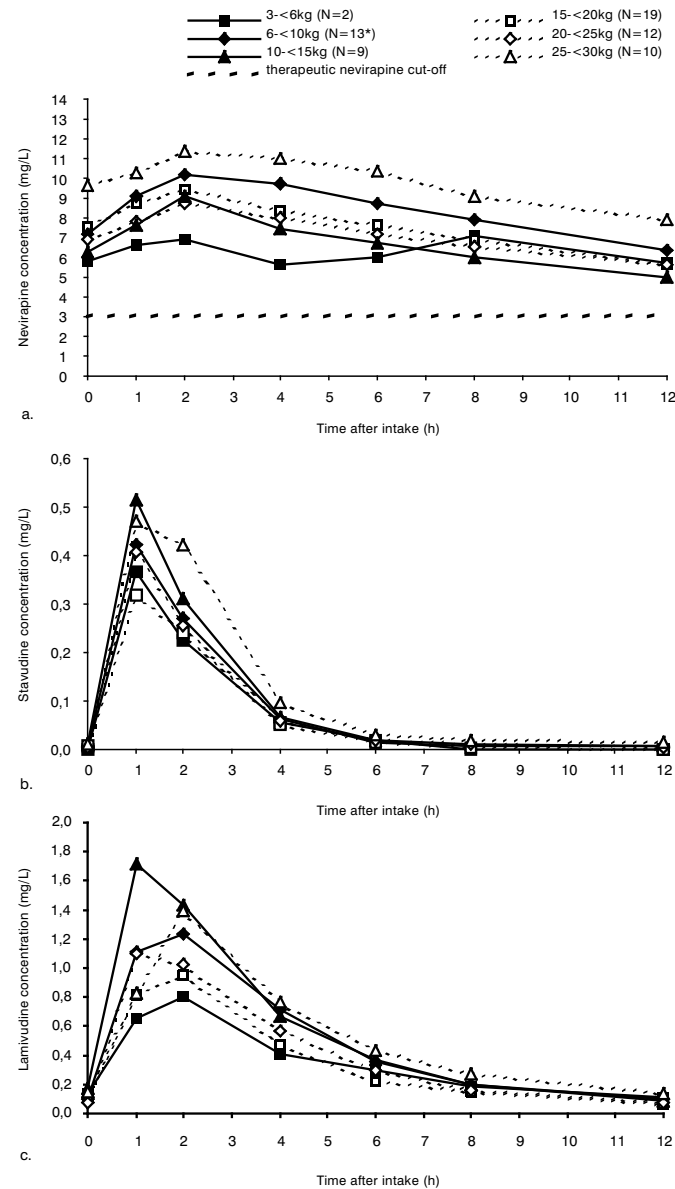
Nevirapine concentrations were higher than those previously reported in adults (Table 3), though C<sub>12h</sub> was subtherapeutic (<3.0 mg/L) [4, 14] in four (6%) children: one in each of the weight-bands 3-<6kg (1 of 2 children), 10-<15kg (1 of 9), 20-<25kg (1 of 12) and 25-<30kg (1 of 10); aged 9 months, 6, 10 and 10 years; and

**Table 2** Baseline demographics and ARV therapy dosing by baseline weight.

	Weight-band, kg						All (n=65)
	3-<6 (n=2)	6-<10 (n=13)	10-<15 (n=9)	15-<20 (n=19)	20-<25 (n=12)	25-<30 (n=10)	
Sex, female	2 (100%)	4 (31%)	5 (56%)	6 (32%)	4 (33%)	3 (30%)	24 (37%)
Age, years	0.8 (0.7, 0.8)	1.3 (0.5, 4.2)	5.2 (2.2, 6.8)	7.0 (4.8, 12.6)	10.2 (7.7, 13.5)	12.9 (10.2, 14.9)	6.9 (0.5, 14.9)
Weight, kg	4.3 (3.4, 5.2)	7.7 (6.0, 9.8)	11.2 (10.0, 13.0)	16.5 (15.0, 19.0)	21.5 (20.0, 24.0)	27.0 (25.0, 29.0)	16.0 (3.4, 29.0)
Weight-for-age z-score	-7.0 (-9.5, -4.6)	-3.7 (-5.8, -1.3)	-3.8 (-7.0, -1.4)	-3.1 (-6.2, -1.2)	-2.8 (-5.0, -0.9)	-3.0 (-4.3, -1.1)	-3.4 (-9.5, -0.9)
Height-for-age z-score	-6.1 (-6.4, -5.8)	-4.1 (-6.5, -1.9)	-3.6 (-5.6, -2.1)	-3.0 (-5.6, -1.3)	-2.1 (-4.1, -0.7)	-2.2 (-4.3, -1.4)	-3.2 (-6.5, -0.7)
BMI-for-age z-score	-4.7 (-9.3, -0.2)	-1.1 (-2.2, +1.4)	-0.4 (-3.6, +1.1)	-0.9 (-4.3, +0.3)	-1.3 (-5.0, -0.3)	-1.9 (-5.9, 0.0)	-1.2 (-9.3, +1.4)
CD4-for-age z-score	-2.7 (-3.3, -2.1)	-2.5 (-5.0, +0.6)	-4.3 (-8.9, -1.0)	-5.4 (-14.7, -0.5)	-5.0 (-8.8, -2.3)	-5.3 (-15.4, -1.7)	-4.3 (-15.4, +0.6)
CD4%	23 (22, 23)	14 (3, 27)	13 (2, 24)	15 (1, 38)	12 (2, 22)	12 (4, 20)	13 (1, 38)
WHO stage	1 (50%)	11 (85%)	9 (100%)	18 (95%)	10 (83%)	8 (80%)	57 (88%)
3	1 (50%)	2 (15%)	0 (0%)	1 (5%)	1 (8%)	2 (20%)	7 (11%)
4							
Daily prescribed nevirapine dose, mg/m <sup>2</sup>	391 (355, 427)	415 (349, 486)	376 (326, 435)	358 (317, 391)	353 (326, 372)	405 (372, 421)	370 (317, 486)
Daily prescribed stavudine dose, mg/kg	2.9 (2.3, 3.5)	2.5 (1.9, 3.0)	2.2 (1.8, 2.4)	1.8 (1.6, 2.0)	1.7 (1.5, 1.8)	1.8 (1.7, 1.9)	1.8 (1.5, 3.5)
Daily prescribed lamivudine dose, mg/kg	14.6 (11.5, 17.6)	12.3 (9.5, 15.0)	10.9 (9.2, 12.0)	9.1 (7.9, 10.0)	8.6 (7.5, 9.0)	8.9 (8.3, 9.6)	9.2 (7.5, 17.6)

Values are n (%) for categorical variables and median (range) for continuous variables.

**Figure 1** Mean (a) nevirapine, (b) stavudine and (c) lamivudine concentrations (mg/L) by weight-band.



\* Stavudine and lamivudine analysis failed for one child in 6-<10kg weight-band.

**Table 3** Pharmacokinetic parameters of nevirapine, stavudine and lamivudine.

	CHAPAS1 study*						Literature data adults**
	3-<6 (n=2)	6-<10 (n=13***)	10-<15 (n=9)	15-<20 (n=19)	20-<25 (n=12)	25-<30 (n=10)	
Nevirapine							
C <sub>12h</sub> (mg/L)	5.7 (1.8, 9.7)	6.4 (3.5, 16.9)	5.0 (2.2, 10.5)	5.6 (3.2, 8.9)	5.6 (2.9, 11.0)	7.9 (1.4, 14.9)	3.7
C <sub>max</sub> (mg/L)	8.5 (5.3, 11.7)	10.7 (5.6, 22.5)	9.1 (4.1, 16.8)	9.6 (6.5, 15.3)	9.0 (4.1, 17.6)	12.0 (3.8, 21.2)	5.7
AUC <sub>12h</sub> (h.mg/L)	76.1 (40.4, 112)	102 (55.3, 232)	80.8 (42.0, 158)	90.6 (61.3, 141)	85.9 (41.3, 169)	118 (32.1, 208)	54.5
Stavudine							
C <sub>12h</sub> (mg/L)	<0.015 (<0.015, <0.015)	<0.015 (<0.015, 0.03)	<0.015 (<0.015, 0.03)	<0.015 (<0.015, 0.03)	<0.015 (<0.015, 0.02)	0.02 (<0.015, 0.11)	0.009
C <sub>max</sub> (mg/L)	0.38 (0.09, 0.68)	0.44 (0.29, 0.89)	0.53 (0.21, 0.84)	0.37 (0.19, 0.53)	0.43 (0.33, 0.64)	0.57 (0.39, 0.82)	0.54
AUC <sub>12h</sub> (h.mg/L)	0.86 (0.40, 1.31)	1.04 (0.58, 2.16)	1.20 (0.54, 1.46)	0.84 (0.35, 1.49)	1.00 (0.73, 1.69)	1.44 (0.79, 2.13)	1.28
Lamivudine							
C <sub>12h</sub> (mg/L)	0.11 (0.07, 0.15)	0.09 (0.05, 0.17)	0.09 (<0.05, 0.15)	0.07 (<0.05, 0.20)	0.08 (<0.05, 0.17)	0.13 (0.06, 0.23)	0.09
C <sub>max</sub> (mg/L)	0.81 (0.20, 1.42)	1.42 (0.62, 2.23)	1.86 (0.69, 3.42)	1.04 (0.34, 2.68)	1.28 (0.86, 2.52)	1.47 (0.88, 2.74)	1.2
AUC <sub>12h</sub> (h.mg/L)	4.14 (1.59, 6.69)	5.94 (3.28, 10.28)	6.76 (4.15, 10.16)	4.26 (1.80, 11.45)	5.08 (3.21, 8.04)	6.45 (3.65, 9.39)	4.7

\* Values are mean (range) [SD].  
\*\* Values are median for nevirapine and mean for stavudine and lamivudine [16, 24, 25].  
\*\*\* Stavudine and lamivudine analysis failed for one child in 6-<10kg weight-band.



receiving daily prescribed nevirapine doses of 326-409 mg/m<sup>2</sup>. Further, variability in nevirapine C<sub>12h</sub> (interquartile range 4.1, 7.0 mg/L) was greater than previously reported in adults (3.2, 5.1 mg/L) [15, 16]. PK parameters of stavudine and lamivudine were comparable to those previously reported in adults (Table 3).

Mean plasma concentrations are shown in Figure 1 by weight-band. As there were only two children in the lowest weight-band 3-<6kg, and one (5.2kg) had low plasma concentrations of nevirapine but high concentrations of stavudine and lamivudine, compared to the other (3.4kg), they were excluded from the statistical analyses. There was no evidence of a difference in nevirapine AUC<sub>12h</sub> across the five remaining weight-bands (p=0.2) or the four age groups (p=0.1). There was a difference in stavudine and lamivudine AUC<sub>12h</sub> by weight-band (p=0.0003 and 0.01 respectively) which appeared to be driven by lower concentrations in the single weight-band with a lower dose in the morning than the evening (15-<20kg, 19 children) (Table 3). There was no significant difference in stavudine or lamivudine AUC<sub>12h</sub> by age group (p=0.6 and 0.4 respectively).

All but three of the children were on co-trimoxazole prophylaxis. Eleven (17%) children took one, one took two and one took three additional medications on the PK day (five amoxicillin, seven ferrous sulphate, three paracetamol, one pyrimethamine/sulfadoxine).

Considering adverse events likely related to nevirapine by ≤ 8 weeks after initiating ARV therapy, three of the 65 children in the substudy had grade 2 nevirapine rashes within one month, which all resolved after 9-11 days following temporary discontinuation of nevirapine (subsequently continued). One further child had a grade 1 nevirapine rash at two weeks, which resolved after seven days, followed by transient grade 3 raised liver enzymes on PK day (single values, then returned to normal); no changes were made to nevirapine. Four other children had transient single measurement grade 3 raised liver enzymes on PK day which returned to normal without nevirapine interruption. Nevirapine PK parameters in the eight children with nevirapine-related toxicities were comparable to those in the 57 without (mean (range) C<sub>max</sub> 10.6 (4.1, 18.9) vs. 9.9 (3.8, 22.5) mg/L, p=0.7; AUC<sub>12h</sub> 102 (40.4, 200) vs. 93.3 (32.1, 232) h.mg/L, p=0.6). In addition, transient asymptomatic laboratory abnormalities not judged related to any ARV drug by ≤ 8

weeks were detected in a small number of children (grade 3 anaemia (n=3), bilirubin (n=1); grade 4 bilirubin (n=1), creatinine (n=1), thrombocytopenia (n=1); all single measurements). One further child had grade 4 bilirubin at week four which was not known to have resolved by death from pneumonia (HIV-related) at nine weeks.

## Discussion

In Zambian HIV-infected children receiving the recently developed paediatric FDCs Triomune Baby or Junior, nevirapine plasma concentrations were higher and more variable than historical data in adults, while overall PK parameters of stavudine and lamivudine were comparable.

Triomune Baby and Junior are small, dispersible, crushable, scored tablets with ARV ratios designed to best fit recommended paediatric doses of individual drugs according to WHO guidelines [6]. Importantly, the nevirapine dose in these FDCs has been increased relative to the dose in the adult FDCs (Triomune) to compensate for higher metabolism of nevirapine in children. We developed weight-band-based dosing tables for Triomune Baby and Junior to simplify dosing in resource-limited settings. We aimed to achieve daily nevirapine doses ≥ 300 mg/m<sup>2</sup>, converted into weight-bands, to minimise the risk of subtherapeutic plasma concentrations [3, 14] and virological failure [5].

Since no paediatric reference drug concentrations are available for these ARV doses and ratios, we compared our results with adult reference values. In these 65 children, nevirapine plasma concentrations were higher than in adults. However, nevirapine C<sub>12h</sub> was subtherapeutic (<3.0 mg/L) [4, 14] in 6% of children and the inter-patient variability was greater than in adults [15]. Therefore we would not recommend a lower nevirapine dose in children, who are at greater risk of underdosing for several reasons [17]. Further, in spite of higher average exposure, nevirapine-related adverse reactions were transient with only temporary treatment interruptions, and nevirapine C<sub>max</sub> and AUC<sub>12h</sub> were comparable between children with and without nevirapine-related toxicities. This reflects the lack of a well-defined upper limit of the therapeutic range. The higher average nevirapine exposure minimised the percentage of children with subtherapeutic concentrations and



therefore the related risk for the development of resistance, without any evidence of increased toxicity. There was no evidence of a difference in nevirapine AUC<sub>12h</sub> by weight-band  $\geq 6$ kg or age group.

We determined stavudine and lamivudine plasma concentrations as surrogates of intracellular concentrations of the pharmacologically active triphosphate metabolites, because obtaining adequate blood volumes for determination of intracellular concentrations of nucleoside reverse transcriptase inhibitor triphosphates is ethically, logistically and technically difficult in children [18]. Our assumption was that comparable plasma concentrations between children and adults would reflect comparable intracellular concentrations. Unlike nevirapine, the plasma concentration parameters of stavudine and lamivudine were comparable to those reported in adults, with no differences across pre-specified age groups. A previous study reported that recommended daily lamivudine dosing (8 mg/kg) [6] leads to lower AUC<sub>12h</sub> and C<sub>max</sub> in younger compared to older children [19]. However, of note, in our study younger children received relatively higher lamivudine doses per kg compared to older children, as a consequence of optimising dosing of three ARVs across six weight-bands, resulting in adequate plasma exposures across all ages.

There was a difference in stavudine and lamivudine AUC<sub>12h</sub> by weight-band, which appeared to be driven by the single weight-band with unequal dosing. However, the reduced plasma exposure to stavudine and lamivudine after the lower morning dose would not be representative of the average 24-hour exposure; in addition the long intracellular half-life of lamivudine may not be reflected in plasma concentrations. Nevirapine exposure appears to be less affected by unequal dosing, most likely because of its long elimination half-life. Although unequal dosing should be avoided where possible for drugs with a short elimination half-life, this may be preferable to increasing complexity and hence risking lower adherence by administering larger numbers of lower dose tablets equally per dosing interval. We plan to investigate adherence, in particular between equal and unequal dosed weight-bands, using detailed adherence data collected in CHAPAS1.

WHO recently recommended Triomune 30 for all adults irrespective of body size because of increasing concerns about stavudine-associated lipodystrophy and data suggesting that the lower dose may be adequate in adults. It is particularly

important not to overdose stavudine in young children who will have long-term exposure to ARVs by virtue of therapy initiation early in life. However, when using stavudine as part of a FDC, it is also crucial not to underdose nevirapine and lamivudine, which have low genetic barriers to resistance. Our previous study in 127 HIV-infected Malawian and Zambian children receiving divided adult Triomune 30 or 40 (30 or 40mg stavudine respectively) demonstrated that it is impossible to achieve adequate nevirapine concentrations in young children without risking overdosing with stavudine, especially when using Triomune 40, but even when using Triomune 30 [3]. In that study, 18% of children had a subtherapeutic random nevirapine plasma concentration ( $<3.0$  mg/L), compared to only 6% with a subtherapeutic trough nevirapine plasma concentration in this current study. Further, the difference in the proportion of subtherapeutic nevirapine concentrations may have been larger if we had evaluated nevirapine C<sub>12h</sub> in our previous study, though smaller if we had directly observed medication intake in that study. In a Thai study, only 3% (1 of 34) of children who received divided adult FDCs (GPO-VIR S30, GPO, Thailand; equivalent to Triomune 30) had subtherapeutic nevirapine concentrations (defined as  $<3.4$  mg/L) [20] and the average nevirapine dose was considerably higher at 328 mg/m<sup>2</sup> [20] compared to 265 mg/m<sup>2</sup> in our previous study [3]. However, the average age was higher and none were  $<3$  years (compared with 13% in our previous study), nor did any receive quarter or three-quarter tablets (compared with 42%) [3].

To avoid overdosing with stavudine while aiming to achieve therapeutic nevirapine concentrations in children taking adult FDCs, liquid formulations allow for more accurate dosing of the smallest children where there are no alternatives [21]. However, liquid formulations are costly, difficult to transport and store, and are complicated for carers to administer (for example, three syrups each of different volume rather than low numbers of whole or divided FDCs). Further, frequent changes in volumes of separate liquid ARVs as a child grows presents considerable challenges for both carers and medical providers. Paediatric FDCs in an appropriate ratio for children, such as Triomune Baby and Junior, are attractive alternatives, especially in resource-limited settings. It is hoped that in the future alternative FDCs, such as Atripla, Truvada and Kivexa, which do not contain stavudine, will become available for children. One disadvantage of FDCs is less flexibility of dosing, exemplified by the common practice of dose escalation of the auto-inducing drug nevirapine during the first two weeks of treatment to avoid high plasma

concentrations and possible toxicity. In the case of Triomune Baby and Junior, this means provision of an additional stavudine/lamivudine tablet during the first two weeks to allow full dosing of these ARVs. CHAPAS1 is currently investigating whether increased complexity of dose escalation outweighs the possible reduction of adverse events. Of note, nevirapine dose escalation in 52% of children in this PK substudy would not have influenced our results as sampling was carried out at steady state [22] and this was confirmed by sensitivity analyses unadjusted for randomisation (results not shown). Our PK data suggest no effect of drug concentrations on the rate of adverse events. Co-medications used in our study would not be expected to influence outcomes.

We assessed the PK of the antiretroviral drugs based on observed intake. Six (of 71, 8%) children did not meet our strict adherence criteria; this raises potential cause for concern regarding adherence, which we will investigate further in our planned adherence analyses. Exclusion of these children allowed reliable estimation of the PK parameters.

Since there were only two children in the lowest weight-band (3-<6kg), there are insufficient data to draw conclusions about dosing for children weighing <6kg at this stage. In light of critical interim results from the CHER trial [23] suggesting the importance of early treatment of infants, this extension to children <6kg is crucial. We are currently enrolling into a further substudy to estimate 4-sample PK curves of the three ARVs in children aged  $\geq 1$  month weighing 3-<6kg at enrollment who are receiving Triomune Baby.

The children in our PK substudy will continue to be followed in CHAPAS1. Further analyses are planned to evaluate possible predictors of ARV plasma concentrations, in particular the impact of malnutrition; we previously demonstrated lower nevirapine concentrations in stunted African children and higher concentrations in wasted children for the same dose per BSA [3]. In addition, analyses are planned to investigate the impact of predictors on subsequent changes in CD4%, viral load and resistance, in particular with respect to the lower plasma exposure of stavudine and lamivudine observed in the weight-band with unequal dosing.

We conclude that the ARV ratio of nevirapine, stavudine and lamivudine in the paediatric FDCs Triomune Baby and Junior is appropriate for children  $\geq 6$ kg;

additional PK sampling is needed in children <6kg. Following successful review of this study in African HIV-infected children, who are a key target population, WHO now recommend the Triomune Baby and Junior ratios and these FDCs have been approved by the United States Food and Drug Administration. It is hoped they will now be made accessible to national ARV therapy programs at low cost, and that future efforts will be made to develop other solid paediatric FDCs which do not contain stavudine.

## Acknowledgements and sources of support

The authors would like to thank the families and children, and staff from the University Teaching Hospital and School of Medicine, Lusaka, Zambia. Technicians from the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands are kindly acknowledged for the analysis of the plasma ARV concentrations. Marthe Le Prevost is acknowledged for help in training on pharmacokinetic techniques for the study. The study medication was supplied by Cipla Pharmaceuticals, India. The CHAPAS1 study was funded by the European and Developing Countries Clinical Trials Partnership (grant CHINTU 2004.01.H.d2.33011) and sponsored by the Medical Research Council, UK.

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## **Part II**

Clinical pharmacology  
in HIV-infected adults



## Chapter 5

### Therapeutic drug monitoring of nevirapine in resource- limited settings

Rafaëlla L'homme<sup>1,2</sup>, Eva Muro<sup>3</sup>, Jacqueline Droste<sup>1,2</sup>, Liselotte Wolters<sup>3</sup>, Noor van Ewijk-Beneken Kolmer<sup>1,2</sup>, Werner Schimana<sup>3</sup>, David Burger<sup>1,2</sup>

<sup>1</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands; <sup>3</sup> Kilimanjaro Christian Medical Centre, Moshi, Tanzania

*Clinical Infectious Diseases* 2008; in press

## Abstract

**Background:** We developed a simple and inexpensive thin layer chromatography (TLC) assay for semi-quantitative detection of nevirapine saliva concentrations in resource-limited settings. The method was validated in an African target population.

**Methods:** Paired plasma and saliva nevirapine concentrations were assayed by high-performance liquid chromatography (HPLC); saliva concentrations were also assayed by TLC. The false positive rate was the proportion of subtherapeutic nevirapine saliva and plasma concentrations by HPLC reported as being therapeutic in saliva by TLC. The false negative rate was the proportion of therapeutic nevirapine saliva and plasma concentrations by HPLC reported as subtherapeutic in saliva by TLC. The extent of agreement in TLC readings between 5 technicians and 2 batches of TLC sheets was evaluated.

**Results:** Twenty-five (9%) of 286 African adults had a subtherapeutic plasma nevirapine concentration. The median saliva/plasma nevirapine concentration ratio was 0.51. The false positive rate by TLC was 0.00 (none out of 23) compared to HPLC saliva result and 0.08 (2 out of 25) compared to HPLC plasma result. The false negative rate by TLC was 0.01 (3 out of 263) compared to HPLC saliva result and 0.01 (3 out of 261) compared to HPLC plasma result. The extent of agreement in TLC result was substantial between 5 technicians (Fleiss' kappa 0.77) and 2 batches of sheets (Cohen's kappa 0.80).

**Conclusions:** The TLC assay was found to be sensitive, specific and robust in detection of subtherapeutic nevirapine concentrations in saliva of African HIV-infected adults. It is an attractive alternative to HPLC for therapeutic drug monitoring of nevirapine in resource-limited settings.

## Introduction

Nevirapine is widely prescribed in combination with nucleoside reverse transcriptase inhibitors for the treatment of HIV-infection in resource-limited countries. Adequate plasma concentrations of nevirapine are required to achieve a successful response, whereas subtherapeutic concentrations (defined as  $<3.0\text{mg/L}$ ) are related to development of mutations and virological failure [1-3]. Even in the case of perfect adherence to standard doses, some patients will remain at risk for underdosing because of interpatient variability in nevirapine exposure or drug-drug interactions.

In the developed world, Therapeutic Drug Monitoring (TDM) [3] is a well-known tool for the optimization of nevirapine dosing in HIV-infected patients. Due to the lack of simple and affordable methods to determine the exposure to nevirapine, TDM is hardly ever performed in resource-limited settings. Previous studies suggest that saliva may be used as an alternative body fluid for TDM of nevirapine [4, 5]. Saliva nevirapine concentrations in HIV-infected [4] and healthy [5] Caucasians are approximately half of the values observed in plasma. We recently developed a thin layer chromatography (TLC) method to provide a simple and economical tool for semi-quantitative measurement of nevirapine in saliva.

The primary objective of this study was to validate our newly developed TLC method for TDM of nevirapine in saliva of HIV-infected Africans. Secondary objectives were to determine the relation between saliva and plasma nevirapine concentrations and the proportion of subtherapeutic nevirapine concentrations in an African population.

## Methods

### Study population

Three hundred HIV-infected adults on a nevirapine (Viramune® or a generic formulation) containing regimen for at least 4 weeks were eligible for enrolment at a routine visit to the adult HIV clinic of the Kilimanjaro Christian Medical Centre (KCMC), Moshi, Tanzania. Patients suffering from oral lesions or ulcers and those who were unable to self-report date and time of last nevirapine ingestion were excluded. The protocol

**Figure 1** Preparation of thin layer chromatography sheet with saliva samples.



was reviewed and approved by the Ethics Committee of Tumbaini University, Moshi, Tanzania. Written informed consent was obtained from all subjects before enrolment.

#### Sample collection and drug concentration assays

At a routine visit to the clinic, unannounced paired blood and saliva samples were collected within 5 minutes. Stimulated saliva was obtained by a salivette (Sartstedt, Etten-Leur, the Netherlands) using a dental cotton roll impregnated with citric acid (20 mg), which stimulates the salivary flow. Study subjects were asked to chew on the roll for approximately 1 minute. Blood and saliva samples were stored at 2-8°C for a maximum of 8 hours. Plasma was separated and stored at -80°C until transportation to the Netherlands on dry-ice. Saliva was obtained by centrifugation of the cotton roll at 800 g for 10 minutes. Two aliquots were stored at -80°C: one for analysis by TLC in Tanzania and one for transportation to the Netherlands and analysis by high-performance liquid chromatography (HPLC).

Plasma and saliva concentrations of nevirapine were assayed at the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, using validated HPLC assays with ultraviolet (UV) detection, modified from a method published by Hollanders et al [6]. Pre-treatment of plasma samples remained the same [6] and did not differ from the preparation of saliva samples. Briefly, 150 µL of saliva or plasma was mixed with 150 µL of perchloric acid, vortexed for 20 seconds and centrifuged for 5 minutes at 11,000 rpm. Subsequently, 200 µL of the clear supernatant was transferred to insert vials and placed in the autosampler. The lower and upper limits of quantification of the modified assays were 0.167 and 16.7 mg/L for plasma and 0.158 and 15.8 mg/L for

saliva. The intraday precision of the assays ranged from 0.4% to 3.2% for plasma and from 1.3% to 4.1% for saliva. Additional variation as a result of performing the assays on different days ranged from 0.0% to 0.4% for plasma and from 0.8% to 2.2% for saliva. The accuracy of the assays ranged from 102% to 105% for plasma and from 99% to 102% for saliva. Saliva/plasma ratios of nevirapine concentrations and the proportion of subjects with a subtherapeutic nevirapine plasma concentration below 3.0 mg/L [3] were determined. In addition, saliva concentrations of nevirapine were semi-quantitatively analysed at the Biotechnology Laboratory of KCMC, Moshi, Tanzania, using a newly developed TLC method.

#### Experimental TLC method

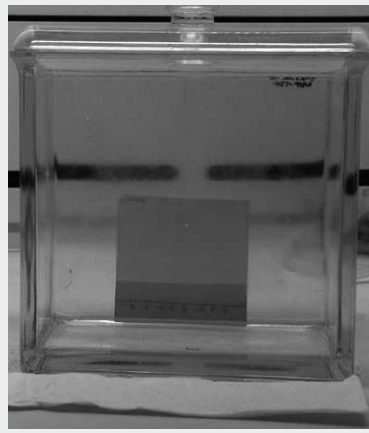
A reference solution of 1.75 mg/L nevirapine (Boehringer Ingelheim) was obtained by dilution of a stock solution (0.875 mg/mL nevirapine in dimethylsulfoxide, DMSO, Merck 102931) in blank saliva; both were kept at -80°C.

The reference solution and saliva samples were thawed at room temperature, mixed (1 minute) and centrifuged (9000 g, 1 minute). Of the reference and samples, 1.0 mL was transferred into a 10 mL glass test tube. After addition of 0.5 mL 0.2 M ammonia (diluted from 25% ammonia, Labopharma) and 5 mL tert-butylmethylether (Riedel - de Haën), tubes were closed, mixed (1 minute), centrifuged (1150 g, 5 minutes) and kept at -80 °C until the lower layer was frozen completely (20 minutes). The organic layer was poured into a 10 mL glass test tube and was dried in two days.

An eluent (or mobile phase) was prepared (toluene:ethyl acetate = 1:1, ACME Chemicals and Unilab, respectively) and poured into a TLC container. The container was closed and left for 1 hour. After addition of 50 µL methanol to the dried test tubes, they were closed and mixed (1 minute). One µL of the reference and samples was slowly pipetted in small dense dots at least 1.5 cm from the side and 2.0 cm from the bottom of a silica gel TLC sheet (height 10.0 cm; width 20.0 cm, Merck), as shown in Figure 1. After drying, spots were checked for similarity of size and shape under UV light (254 nm, CAMAG 022.9120). The TLC sheet was placed in the container with eluent for approximately 12 minutes (Figure 2). It was marked for the distance the eluent had moved and was dried in the air. The intensity of the spots was determined under UV light and compared to the reference spot (Figure 3). A spot that was less intense than the reference was considered subtherapeutic (see



**Figure 2** Thin layer chromatography sheet in container with eluent.



**Figure 3** Determination of spot intensity under ultraviolet light.



below). A spot that was comparable to or more intense than the reference spot was considered therapeutic.

#### Technical validation of TLC method

All saliva samples were used to determine the technical sensitivity and specificity of the TLC method. The readings were performed by one technician. Saliva nevirapine concentrations by HPLC were defined as subtherapeutic if below 1.5 mg/L, which is half of the cut-off value for plasma (3.0 mg/L [3]). The false positive rate was defined as the proportion of subtherapeutic nevirapine saliva concentrations by

HPLC [6] (<1.5 mg/L) that was reported as therapeutic in saliva by TLC. The false negative rate was defined as the proportion of therapeutic nevirapine saliva concentrations by HPLC [6] ( $\geq 1.5$  mg/L) that was reported as subtherapeutic in saliva by TLC.

Twenty-five saliva samples with nevirapine concentrations by HPLC [6] closest to the TLC reference were selected to test the robustness of the TLC method. The extent of agreement in TLC result between 5 different technicians (Fleiss' kappa [7]) and 2 different batches of TLC sheets (Cohen's kappa [8]) was evaluated in these 25 saliva samples.

The stability of the stock solution (nevirapine in DMSO) was tested after storage at -40 °C for 50 months. The stability of the reference solution (nevirapine in blank saliva) was tested after storage at room temperature for 17 hours, after storage at -40 °C for 2 months, and after freezing and thawing twice. Interference of possible co-medications was evaluated by comparison of the retention factor (Rf: distance travelled by compound divided by distance travelled by eluent front) of extractable and detectable (UV light, 254 nm) compounds with the Rf value of nevirapine. In addition, blank saliva samples from at least 6 subjects not taking nevirapine were tested for interference by endogenous substances.

#### Biological validation of TLC method

All plasma and saliva samples were used to determine the biological sensitivity and specificity of the TLC method. The false positive rate was defined as the proportion of subtherapeutic nevirapine plasma concentrations by HPLC [6] (<3.0 mg/L [3]) that was reported as therapeutic in saliva by TLC. The false negative rate was defined as the proportion of therapeutic nevirapine plasma concentrations by HPLC [6] ( $\geq 3.0$  mg/L [3]) that was reported as subtherapeutic in saliva by TLC.

## Results

Of the 300 subjects enrolled in the study, 14 were excluded because of problems with labelling, too little volume of saliva or lost saliva. The mean (range) age of the remaining 286 African subjects (200 women) was 41 (17, 71) years. All subjects were



**Table 1** Number of (sub)therapeutic nevirapine concentrations by Thin layer chromatography (TLC), compared to High-performance liquid chromatography (HPLC), sensitivity and specificity of TLC assay.

		TLC - Saliva			
		Subtherapeutic	Therapeutic	Sensitivity	Specificity
HPLC - Saliva	Subtherapeutic	23	0	100%	99%
	Therapeutic	3	260		
HPLC - Plasma	Subtherapeutic	23	2	92%	99%
	Therapeutic	3	258		

Subtherapeutic concentrations were defined as < 1.75 mg/L (saliva, TLC), < 1.5 mg/L (saliva, HPLC) and < 3.0 mg/L (plasma, HPLC). Therapeutic concentrations were defined as higher values than these cut-off values.

treated with a nevirapine dose of 200 mg twice daily (BID) in combination with lamivudine and either stavudine or zidovudine.

The mean (range) plasma nevirapine concentration by HPLC was 7.35 (<0.167, 28.59) mg/L. Twenty-five out of 286 (9%) subjects had a subtherapeutic plasma nevirapine concentration (i.e., below 3.0 mg/L); 18 of 25 were below the detection limit of the HPLC assay (0.167 mg/L). The median (interquartile range, IQR) saliva/plasma nevirapine concentration ratio by HPLC was 0.51 (0.47, 0.54) and the median (IQR) time between last ingestion and sampling was 3.40 (2.52, 4.37) hours. There were six subjects with a saliva/plasma concentration ratio above 1.00 (range 1.25, 15.68); the median (IQR) time between last ingestion and sampling for these 6 subjects was 1.19 (0.85, 1.74) hours.

None of the 23 subtherapeutic (<1.5 mg/L) nevirapine saliva concentrations according to HPLC were reported as therapeutic by TLC (false positive rate of 0.00); the technical sensitivity of the TLC method was 100% (Table 1). Three of the 263 therapeutic ( $\geq 1.5$  mg/L) nevirapine saliva concentrations (1.62, 1.74 and 1.96 mg/L) according to HPLC were reported as subtherapeutic by TLC (false negative rate of 0.01); the technical specificity of the TLC method was 99% (Table 1).

The extent of agreement in TLC result (subtherapeutic or therapeutic nevirapine saliva concentration) of 25 selected samples with a concentration closest to the

reference was substantial between 5 technicians (Fleiss' kappa of 0.77) and 2 batches of TLC sheets (Cohen's kappa of 0.80).

The stock solution for the TLC method (nevirapine in DMSO) was stable at  $-40^{\circ}\text{C}$  for at least 50 months (mean  $\pm$  SD recovery  $99.7 \pm 0.3\%$ ). The reference solution (nevirapine in blank saliva) was stable at room temperature for at least 17 hours (recovery  $107.6 \pm 5.8\%$ ) and at  $-40^{\circ}\text{C}$  for at least 2 months (recovery  $94.3 \pm 3.2\%$ ). Freezing and thawing twice had no effect on the stability of nevirapine in blank saliva (recovery  $95.1 \pm 1.1\%$ ). The average nevirapine Rf value was 0.29; none of the possible co-medications that were extractable and detectable had a similar Rf value. Also, no interference by endogenous substances was observed in the blank saliva samples from subjects not taking nevirapine.

Two of the 25 subtherapeutic (<3.0 mg/L) nevirapine plasma concentrations (2.61 and 2.91 mg/L) according to HPLC were reported as therapeutic by TLC (false positive rate of 0.08); the biological sensitivity of the TLC method was 92% (Table 1). Three of the 261 therapeutic ( $\geq 3.0$  mg/L) nevirapine plasma concentrations (3.41, 3.52 and 4.28 mg/L) according to HPLC were reported as subtherapeutic by TLC (false negative rate of 0.01); the biological specificity of the TLC method was 99% (Table 1).

## Discussion

Twenty-five out of 286 (9%) HIV-infected African adults had a subtherapeutic nevirapine plasma concentration <3.0 mg/L [3]. Saliva nevirapine concentrations were approximately half of the values observed in plasma. A TLC method for semi-quantitative detection of nevirapine in saliva was found to be sensitive, specific and robust. This simple and economical tool is a good option for TDM of nevirapine in resource-limited settings.

Using saliva instead of plasma for TDM of nevirapine implies painless and non-invasive sampling with diminished risk of HIV-transmission to health care workers at a lower cost [4]. TLC is a relatively inexpensive assay technique compared to HPLC [9], which is commonly used in the developed world for TDM of

nevirapine in plasma. An HPLC system for TDM of nevirapine costs approximately €40,000 and the costs for consumables per sample are estimated at €15. In contrast, the estimated initial set-up cost of a new TLC method for semi-quantitative measurement of nevirapine in saliva are €800 and the costs for consumables per sample are approximately €1.60. Since this simple and inexpensive method was developed to perform TDM of nevirapine in resource-limited settings, it was validated in an African target population in the current study.

The percentage of HIV-infected adults with a subtherapeutic nevirapine plasma concentration <3.0 mg/L [3] according to HPLC [6] in our Tanzanian population (9%) was low compared to the percentage in a previous report from Malawi (16%) [10]. This may be due to the unreliable drug supply during the time of the Malawian study (2003), which explained more than one-third of the total reported non-adherence [10]. The fact that 18 of the 25 subtherapeutic nevirapine plasma concentrations in our Tanzanian population were undetectable indicates non-adherence, since nevirapine is generally detected up to weeks after termination of drug intake due to its long elimination half-life [11].

The median saliva/plasma nevirapine concentration ratio of 0.51 according to HPLC [6] in our population of HIV-infected Africans was comparable to the ratios observed in HIV-infected [4] and healthy [5] Caucasians. It has been suggested that thorough rinsing of the mouth is required prior to saliva sampling, because remnants of orally administered medicines may contaminate saliva specimens and yield spuriously high values [12]. Indeed, 6 of the 286 subjects in the current study had an unexpectedly high saliva/plasma nevirapine concentration ratio above 1 [4, 12], which may be due to chewing tablets without rinsing the mouth prior to sampling. This explanation is supported by our observation that the time between last ingestion and sampling was shorter for those subjects with an unexpectedly high nevirapine concentration in saliva compared to plasma.

Since saliva nevirapine concentrations by HPLC were approximately half of the values observed in plasma, they were defined as subtherapeutic if below 1.5 mg/L rather than below the cut-off value for plasma of 3.0 mg/L [3]. The main requirement of our TLC method for semi-quantitative detection of nevirapine in saliva was appropriate sensitivity, meaning detection of nevirapine concentrations that were

subtherapeutic according to HPLC, in order to pick out those subjects at risk for nevirapine resistance. During the development of our TLC method, subtherapeutic saliva concentrations just below 1.5 mg/L were sufficiently distinguishable from a TLC reference of 1.75 mg/L, but not from a reference of 1.5 mg/L. For the validation of our TLC method we have therefore chosen a reference of 1.75 mg/L, which has led to an excellent technical sensitivity (100%) and specificity (99%). None of the subtherapeutic nevirapine saliva concentrations according to HPLC were reported as therapeutic by TLC and the few therapeutic saliva concentrations that were reported as subtherapeutic by TLC were all close to the cut-off value for saliva (1.5 mg/L).

The biological validation comparing nevirapine saliva concentrations by TLC to plasma concentrations by HPLC (subtherapeutic or therapeutic) resulted in an acceptable sensitivity (92%) and excellent specificity (99%). False positive and negative results were all close to the cut-off value for plasma (3.0 mg/L). The somewhat lower biological sensitivity can be explained by the fact that some subjects with a subtherapeutic plasma concentration according to HPLC had a therapeutic saliva concentration according to both HPLC and TLC. Although these subjects had a higher saliva/plasma ratio than average, none of them had a ratio above 1.

As expected, it was found to be most difficult to interpret nevirapine saliva concentrations closest to the TLC reference. To test the worst-case scenario robustness of the TLC method we selected 25 samples closest to the reference. The fact that the extent of agreement in TLC results between 5 technicians and 2 batches of TLC sheets was substantial provides an indication that the outcome of our TLC method is reliable during normal use.

At this moment we are planning to roll out the TLC method in various African countries as well as in Indonesia. This will make it possible to further evaluate its robustness and practical application to monitor adherence. Potentially, the assay could be further developed commercially.

In conclusion, we developed a simple and economical TLC method for semi-quantitative detection of nevirapine saliva concentrations. The assay was found to be sensitive, specific and robust in the detection of subtherapeutic nevirapine

concentrations in an African population. It is an attractive alternative to HPLC for therapeutic drug monitoring of nevirapine in resource-limited settings.

## Acknowledgements and sources of support

We would like to thank the patients for their participation in this study. Laizer Leshoki Saitory of the Kilimanjaro Christian Medical Centre (KCMC), Moshi, Tanzania is kindly acknowledged for the screening of study subjects. The study nurses at KCMC are thanked for the collection of study samples. The study was funded by Poverty Related Infection Oriented Research (PRIOR), a research network sponsored by the Netherlands Foundation for the Advancement of Tropical Research (NWO-WOTRO) and the Netherlands Foundation for Health Research and Development (ZonMw).

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## Chapter 6

Enzyme inducers reduce  
elimination half-life after a  
single dose of nevirapine  
in healthy women

Rafaëlla L'homme<sup>1,2</sup>, Tim Dijkema<sup>3</sup>, Andre van der Ven<sup>2,4</sup>, David Burger<sup>1,2</sup>

<sup>1</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands; <sup>3</sup> Clinical Research Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>4</sup> Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

*Journal of Acquired Immune Deficiency Syndromes* 2006; 43:193-6

## Abstract

**Objective:** Single-dose nevirapine to prevent mother-to-child transmission of HIV is associated with development of nevirapine resistance, probably due to its long half-life in combination with a low genetic barrier to resistance. The objective of this study was to find enzyme inducers to reduce nevirapine half-life.

**Design:** The design of this phase-I pharmacokinetic study was single-center, open-label, two-period, nine-group.

**Methods:** After administration of a single 200-mg dose of nevirapine to HIV-seronegative non-pregnant women in both period 1 and 2, blood was sampled twice-a-week for 21 days. In period 2 additional interventions (single dose carbamazepine, phenobarbital or phenytoin; phenytoin for 3 or 7 days; St John's Wort, vitamin A or cholecalciferol for 14 days) were administered to all subjects except for the control group.

**Results:** Thirty-six subjects participated. In three intervention groups T-half ratio (nevirapine half-life in period 2 / half-life in period 1) differed significantly from the control group: single 400-mg dose of carbamazepine ( $P = 0.021$ ), 184mg phenytoin once daily for 3 ( $P = 0.021$ ) or 7 days ( $P = 0.021$ ). The median decrease in nevirapine half-life was 18.8, 19.0 and 16.9 hours, respectively.

**Conclusion:** Interventions with a single dose of 400mg carbamazepine or phenytoin 184mg for 3 or 7 days effectively reduced nevirapine half-life. Appropriately powered safety and feasibility endpoint studies are warranted before these interventions can be tested in the setting of single-dose nevirapine for prevention of mother-to-child transmission of HIV to reduce the development of nevirapine resistance.

## Introduction

Without treatment, the risk to transmit HIV to the child during pregnancy or delivery is approximately 25 to 48%. Complexity and costs of several effective strategies to prevent mother-to-child transmission (MTCT) of HIV presently limit large-scale introduction in low-income countries. However, the administration of a single dose of the antiretroviral drug nevirapine (NVP) to the mother shortly before delivery and to the newborn within the first 24 to 72 hours after birth [1] is simple and affordable for low-income countries. This so-called single dose NVP (SD-NVP) strategy reduces the risk of MTCT by 50%.

Aside from the suboptimal effectiveness of the SD-NVP strategy, an important disadvantage is that in approximately 20 to 70% of the women the virus develops resistance against NVP [2, 3]. This can have a great impact. First, the effectiveness of SD-NVP may be diminished in a following pregnancy. Second, efficacy can be diminished when the mother herself has an indication for NVP-based highly active antiretroviral therapy (HAART) in the future [4]. Third, there is a possibility to transmit resistant HIV to others. NVP resistance can develop due to the long elimination half-life of NVP in combination with a low genetic barrier to resistance. In one study, women who developed the K103N mutation after intake of a single NVP dose had a significantly longer elimination half-life of NVP than those in whom no resistance was detected (74.8 vs. 51.8 hours;  $P = 0.01$ ) [5].

A short-course of an enzyme inducer may prevent the development of resistance by decreasing elimination half-life of NVP. The primary elimination pathway for NVP appears to be the oxidative metabolism by cytochrome P-450 enzymes CYP3A4 and CYP2B6 [6]. Several potent CYP3A inducers have been described in literature. Long-term treatment with anticonvulsants such as carbamazepine, phenobarbital and phenytoin increased the clearance of antipyrine, which is a broad marker of enzyme induction, on average to a similar extent [7]. St John's Wort increased the clearance of NVP with 35% [8]. PXR-mediated up-regulation of CYP3A4/CYP3A7 and CYP3A5 by retinol and  $\beta$ -carotene points to a potential interference on the metabolism of xenobiotic and endogenous relevant compounds [9]. Finally, the fully active dihydroxylated metabolite of cholecalciferol,  $1\alpha,25-(\text{OH})_2\text{D}_3$ , was shown to induce the expression of CYP3A4 and, to a lesser extent, CYP2B6 and CYP2C9 genes in normal differentiated primary human hepatocytes [10].

The primary objective of this pilot study was to investigate the effect of intervention strategies with a number of the above described agents on the elimination half-life of NVP. The study was conducted in the Netherlands as a prelude to studies in Tanzania. Appropriately powered safety and feasibility endpoint studies need to be performed before these interventions can be tested in the setting of SD-NVP for prevention of MTCT (PMTCT) of HIV to reduce the development of nevirapine resistance.

Methods

The present study was a single-center, open-label, two-period, nine-group, phase-I pharmacokinetic study. Non-pregnant healthy women aged 18 to 40 years were eligible for enrollment after pre-entry and laboratory evaluation. Women tested positive for HIV and/or hepatitis B or C were excluded. Subjects were not allowed to take any concomitant drug, including hormonal contraceptives and vitamin supplements (for two weeks preceding dosing), except for paracetamol and loperamid. The study protocol was reviewed and approved by the Ethics Committee of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Informed consent was obtained from all women before enrollment.

The study design is shown in Table 1. In both period 1 and 2, sampling of blood was done just before, 8 hours after and 3, 7, 10, 14, 17, and 21 days after intake of NVP. Plasma samples were stored at -40 °C until analysis. Plasma NVP levels were determined in all samples by validated high-performance liquid chromatography (HPLC) assay with ultraviolet (UV) detection (Thermo, Breda, the Netherlands). The lower and upper limits of quantification were 0.03 and 15 mg/L, respectively. The intra- and interday precision ranged from 0.4% to 11.4% and from 0.0% to 2.1%, respectively. The accuracy of the assay ranged from 100.1% to 104.8%. NVP half-life was calculated per group in both period 1 and 2 using all quantifiable NVP levels. This exploratory pilot study with 4 participants per group was not powered to perform statistical tests.

In period 2, several medication levels were determined for safety reasons. Plasma concentrations of carbamazepine (group 2), phenobarbital (group 3) and phenytoin (group 4-6) were determined in the blood sampled 8 hours after intake on day 0 by

Table 1 Study design.

Period 1*
• Group 1-9: SD NVP 200mg
Period 2*
• Group 1: SD NVP 200mg
• Group 2: SD NVP 200mg + SD carbamazepine 400mg
• Group 3: SD NVP 200mg + SD phenobarbital 200mg
• Group 4: SD NVP 200mg + SD phenytoin 184mg
• Group 5: SD NVP 200mg + phenytoin 184mg, 3 days
• Group 6: SD NVP 200mg + phenytoin 184mg, 7 days
• Group 7: SD NVP 200mg + St John's Wort tea** bid, 14 days
• Group 8: SD NVP 200mg + SD retinyl palmitate 200000 IU + β-carotene 30mg & retinyl palmitate 4000 IU, 13 days
• Group 9: SD NVP 200mg + cholecalciferol 2000 IU, 14 days
* Each group consisted of 4 participants. NVP was taken with water during a standardised breakfast on day 0 of period 1 and 2 (with an interval of 4 weeks). First doses of interventions were taken simultaneously with NVP in period 2.
** One cup (tea bag of 2g St John's Wort & 150mL boiling water, 10 minutes). NVP (nevirapine)

validated immunoassay TDxFLx (Abbott, Amsterdam, the Netherlands). In addition, trough levels of phenytoin were determined in the blood samples of day 3 (group 5-6) and day 7 (group 6). Concentrations of retinol (group 8) and 25(OH)D (group 9) were determined in the blood sampled 8 hours after intake on day 0 by validated HPLC assay. Furthermore, trough levels of retinol (group 8) and 25(OH)D (group 9) were determined in the blood samples of day 7 and 14.

Results

Thirty-six non-pregnant healthy women were enrolled in the protocol. The median age, height, and body weight (interquartile range) were 22 (20-24) years, 1.71 (1.67-1.75) m, and 64.5 (60.0-73.0) kg, respectively. All women were white. One woman randomized to group 7 dropped out of the study during period 1 for personal reasons.

The median elimination half-life of NVP (range) in period 1 was 53.9 (34.2-104.2) hours. Pharmacokinetic parameters of NVP in period 1 and 2 are illustrated per



medication group in Table 2. Non-parametric statistical tests (Mann-Whitney) were performed on T-half ratio (NVP half-life in period 2 divided by NVP half-life in period 1). T-half ratios of three intervention groups differed significantly from group 1 (control): single 400-mg dose of carbamazepine (group 2; P = 0.021), 184mg phenytoin once daily for 3 days (group 5; P = 0.021) and 184mg phenytoin once daily for 7 days (group 6; P = 0.021). In these 3 intervention groups, the median [range] decrease in NVP half-life in period 2 was 18.8 [15.6-38.0], 19.0 [11.4-25.4] and 16.9 [10.9-37.4] hours, respectively. The decrease in NVP half-life has led to faster undetectable NVP levels in group 2, 5 and 6 with carbamazepine single dose, phenytoin 3 and 7 days, respectively. In these 3 intervention groups, the median [range] decrease in time to undetectable NVP levels in period 2 was 4.0 [3.0–7.0], 7.0 [7.0-7.0] and 8.5 [7.0-11.0] days, respectively. There are no large differences between maximum NVP plasma levels eight hours after intake in the two periods.

Eight hours after intake of a single dose, carbamazepine levels in group 2 ranging from 3.78 to 6.03 mg/L were all close to the lowest level of the therapeutic range of 4-10 mg/L. Phenobarbital levels in group 3 eight hours after intake of a single dose ranged from 5.10 to 5.83 mg/L and were thus below the therapeutic range of 10-40 mg/L. Phenytoin levels in group 4-6 ranging from 2.36 to 6.27 mg/L were all below the therapeutic range of 8-20 mg/L. Retinol and 25(OH)D levels were all within the normal range of 0.70-3.00 µmol/L and 25-100 nmol/L, respectively.

One out of 36 women in period 1 and 2 out of 36 women in period 2 showed a mild elevation of the liver enzyme ALAT (84, 95 and 114 U/L, respectively). Two out of 4 women taking carbamazepine and 5 out of 12 women taking phenytoin experienced mild vertigo. All 4 women in the phenobarbital group experienced mild somnolence. Adverse events were transient and did not influence the activities of daily living.

Discussion

The present pilot study shows that NVP elimination half-life can be effectively decreased by interventions with a single 400-mg dose of carbamazepine or 184mg phenytoin for 3 or 7 days, most likely due to enzyme induction.

Table 2 Pharmacokinetic parameters of NVP in period 1 & 2, and values in period 2 minus values in period 1 (median, range).

	NVP half-life, T <sub>1/2</sub> (hours)		Time to first undetectable NVP level, T <sub>undetect</sub> (days)		NVP concentration 8 hours after intake, C <sub>8h</sub> (mg/L)	
	Period 1	Period 2	Period 1	Period 2	Period 1	Period 2
*						
1	48.4 [44.4, 58.9]	46.3 [37.9, 62.5]	15.5 [14.0, 17.0]	14.0 [14.0, 17.0]	2.19 [1.35, 2.24]	2.02 [1.17, 2.78]
2	52.5 [46.9, 104.2]	33.8 [31.3, 66.2]	15.5 [14.0, 28.0]	12.0 [10.0, 21.0]	1.81 [1.71, 2.46]	2.03 [1.73, 2.65]
3	49.4 [34.2-69.4]	39.7 [30.3-48.3]	14.0 [10.0, 21.0]	14.0 [10.0, 14.0]	1.98 [1.88, 2.14]	1.91 [1.67, 2.01]
4	58.5 [35.3, 66.5]	50.4 [31.7, 70.8]	17.0 [10.0, 21.0]	15.5 [10.0, 21.0]	1.89 [1.72, 2.12]	2.00 [1.68, 2.39]
5	46.1 [37.6-59.9]	27.1 [26.1, 34.5]	15.5 [14.0, 21.0]	8.5 [7.0, 14.0]	2.24 [2.02, 2.74]	2.45 [1.99, 3.34]
6	55.3 [37.5, 69.7]	34.5 [26.6, 40.3]	17.0 [14.0, 21.0]	8.5 [7.0, 10.0]	1.83 [1.43, 2.29]	2.05 [1.75, 2.25]
7	52.0 [40.6-79.9]	54.2 [38.2, 74.2]	14.0 [14.0, 21.0]	17.0 [14.0, 21.0]	2.02 [1.34, 2.02]	1.84 [1.24, 1.92]
8	53.0 [46.2, 66.1]	56.1 [44.4, 66.9]	15.5 [14.0, 17.0]	17.0 [14.0, 21.0]	1.96 [1.60, 2.26]	1.94 [1.53, 2.04]
9	62.2 [54.4, 62.8]	54.8 [52.7, 60.8]	17.0 [14.0, 21.0]	17.0 [17.0, 17.0]	2.01 [1.53, 2.52]	1.99 [1.58, 2.79]
* Group. NVP (nevirapine)						

This study with small groups was not designed to perform statistical tests. However, because of large and very consistent decreases in NVP half-life, we decided to perform a non-parametric statistical test on the primary parameter T-half ratio to see whether the effect was actually significant.

The interventions with St John's Wort tea, vitamin A and D for 14 days did not show a decrease in NVP half-life. For St John's Wort this may be explained by the fact that the product used in this study (tea) contains a relatively low amount of hyperforine [11], which is the constituent likely responsible for enzyme induction [12]. For vitamin D, one explanation might be that after intake of cholecalciferol by healthy women without vitamin D deficiency, in vivo conversion to the fully active dihydroxylated metabolite  $1\alpha,25\text{-(OH)}_2\text{D}_3$  is not optimal. A single 200-mg dose of phenobarbital was not enough to substantially reduce NVP half-life. Lengthening treatment would not be wise, because of slow elimination by the nursing infant. It seemed to be necessary to take 184mg of phenytoin once daily for at least three days to significantly reduce NVP half-life. In the African setting of PMTCT it should be safe to use phenytoin during breast feeding [13], because of its poor passage into breast milk. Surprisingly, the simple intervention with a single 400-mg dose of carbamazepine appeared to effectively reduce NVP half-life. It is unknown whether this effect is due to enzyme activation, enzyme induction or the up-regulation of the efflux transporter P-glycoprotein (P-gp) [14], although data being conflicting about NVP being a substrate for P-gp [15]. Carbamazepine passes into breast milk, but is generally considered safe for use during breast feeding [13]. Rifampicin is a potent inducer of CYP3A, decreasing NVP AUC by 37% to 58% [6] and was also considered by us to be used in this pilot study. However, rifampicin was not part of the interventions because widespread use together with SD-NVP may promote the development of resistance against rifampicin in tuberculosis (co-)infected patients.

NVP plasma levels eight hours after intake were not influenced by interventions with enzyme inducers. This is consistent with a delay of the effect on enzyme induction as increased protein synthesis is required and this takes a few days for maximum result. It is unlikely that these interventions will influence the protective effect of NVP on MTCT of HIV during labor, because there was no influence on maximum NVP levels. Since this finding is based on a study with small groups of healthy women, it needs to be confirmed in clinical practice.

It is clear that our study population of healthy non-pregnant Dutch women of child-bearing age is not similar to the setting in sub-Saharan African countries, where HIV-infected pregnant women are black and will have different dietary habits, body weights, and concomitant medications. The CYP2B6 T/T genotype at position 516 is more common in African-Americans than in European-Americans and is associated with greater efavirenz exposure [16] and, to a lesser extent, also to greater NVP exposure [17]. However, the median NVP half-life in our group of 36 white women (53.9 hours) does not differ much from the mean NVP half-life in a smaller group of pregnant HIV-infected Ugandan women receiving SD-NVP (61.3 hours) [18]. The results from this pilot study are valuable, since we were able to identify three potential interventions (single 400-mg dose of carbamazepine, 184mg phenytoin for 3 or 7 days) to be studied in the setting of PMTCT to confirm our finding that NVP half-life will be diminished and to test the hypothesis that hereby development of NVP resistance will decrease. The most rational intervention to start with is a single dose of carbamazepine, because of its simplicity.

Addition of other antiretroviral agents after delivery to cover the window of opportunity for the virus to select for nevirapine resistance is a different approach. Recently, preliminary data were presented that short courses (4-7 days) of zidovudine plus lamivudine (Combivir®) added to SD-NVP in the prevention of MTCT, significantly reduced the development of NVP resistance when compared with no intervention [19]. However, the substantial increase in costs and complexity is not desirable. Recent data from a study in Zambia showed that it is already difficult to accurately carry out the simple SD-NVP intervention [20].

It is currently unknown at what plasma level NVP selects for resistance. NVP levels that are either undetectable or, in the context of HAART, greater than 3.0-3.4 mg/L [21, 22], do not have selective pressure. The intervention with 4-7 days Combivir® covers the potential zone of selective pressure below 3.0-3.4 mg/L only partially, which explains that NVP resistance was not fully absent in the intervention arms [19]. A significant decrease in NVP half-life by addition of an enzyme inducer will reduce the duration of the potential zone of resistance, but may also not be sufficient to prevent development of all NVP mutations. It might be interesting to study if combining these two approaches of decreasing NVP half-life and co-administering other antiretroviral agents will be of additional value.



In conclusion, the interventions with a single 400-mg dose of carbamazepine, 184mg phenytoin once daily for 3 or 7 days effectively reduced NVP half-life after intake of a single 200-mg dose of NVP, leading to faster undetectable NVP levels. Appropriately powered safety and feasibility endpoint studies are warranted before these interventions can be tested in the setting of SD-NVP for PMTCT in Africa to confirm our finding of decreased NVP half-life and to test the hypothesis that hereby the development of NVP resistance will be reduced.

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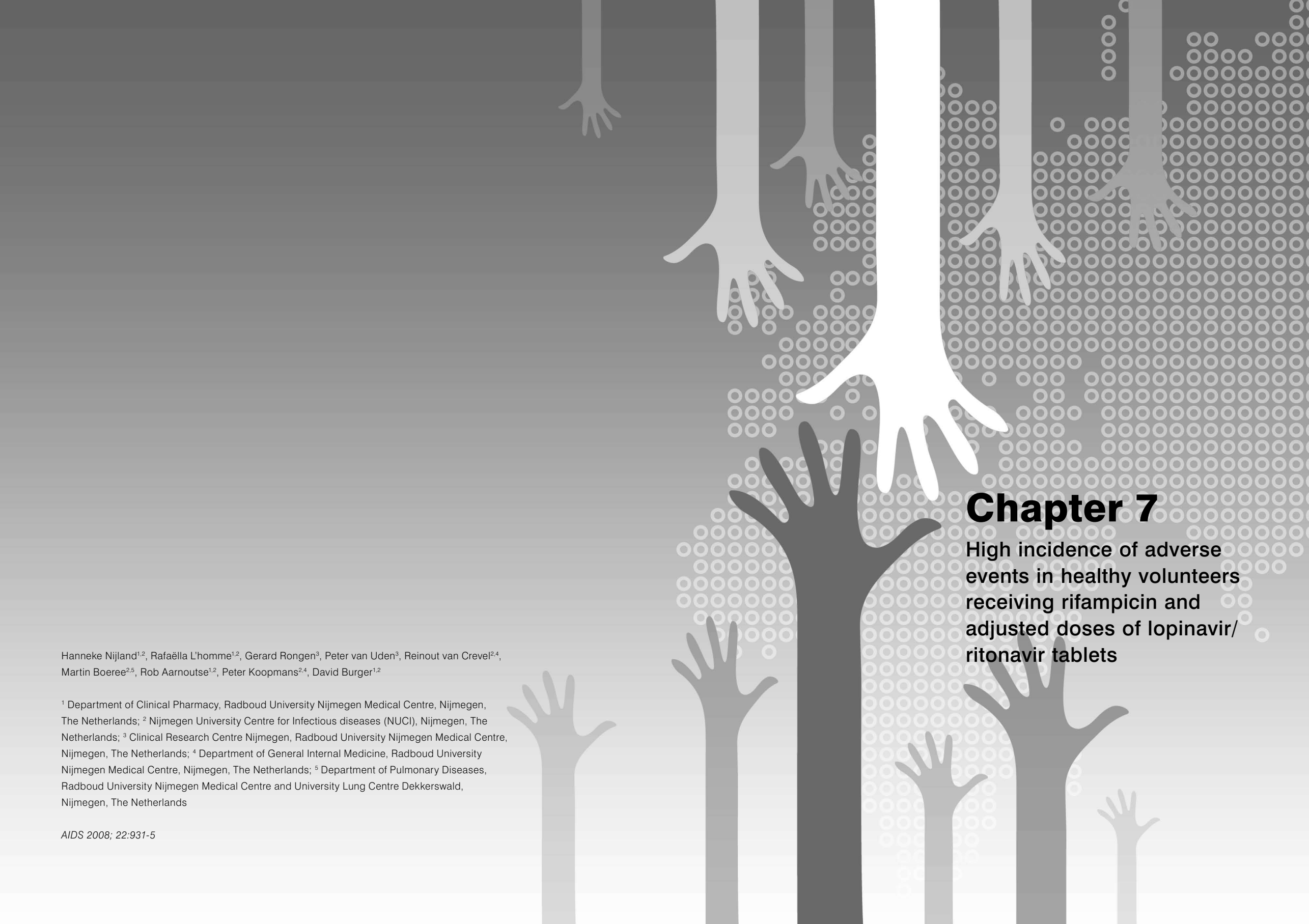
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## **Part III**

Clinical pharmacology  
in HIV-TB co-infection



## Chapter 7

High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets

Hanneke Nijland<sup>1,2</sup>, Rafaëlla L'homme<sup>1,2</sup>, Gerard Rongen<sup>3</sup>, Peter van Uden<sup>3</sup>, Reinout van Crevel<sup>2,4</sup>, Martin Boeree<sup>2,5</sup>, Rob Aarnoutse<sup>1,2</sup>, Peter Koopmans<sup>2,4</sup>, David Burger<sup>1,2</sup>

<sup>1</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands; <sup>3</sup> Clinical Research Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>4</sup> Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>5</sup> Department of Pulmonary Diseases, Radboud University Nijmegen Medical Centre and University Lung Centre Dekkerswald, Nijmegen, The Netherlands

## Abstract

**Objective:** Previous research in healthy volunteers has demonstrated that rifampicin and adjusted doses of lopinavir/ritonavir soft-gel capsules resulted in adequate exposure to lopinavir. Our objective was to study the combined use of rifampicin and the newly introduced lopinavir/ritonavir tablets.

**Methods:** A total of 40 healthy subjects was planned to start with 600 mg rifampicin once daily from day 1–5. From days 6–15, subjects were randomized to receive lopinavir/ritonavir tablets dosed as either 600/150 mg or 800/200 mg twice daily, both in addition to 600 mg rifampicin once daily. A 12h pharmacokinetic curve was planned on day 15. Safety assessments were conducted regularly throughout the study period.

**Results:** Eleven subjects started as the first group in this study. No major complaints occurred during day 1–5 (rifampicin only). After addition of lopinavir/ritonavir, eight subjects suffered from both nausea and vomiting, one from nausea only, and one from vomiting only. On day 7 increases in AST/ALT levels were reported in all subjects and on day 8 the study was prematurely terminated. The AST/ALT levels continued to rise and peaked (grade 2: n=2, grade 3: n=1, grade 4: n=8) on days 9–10. All values returned to normal within six weeks.

**Conclusions:** This study showed a high incidence of adverse events when a higher than standard dose of the new lopinavir/ritonavir tablets was combined with rifampicin. In the future, this drug combination should not be given to healthy volunteers. Liver function should be carefully monitored when rifampicin and lopinavir/ritonavir are combined in patients.

## Introduction

Approximately one-third of the 40 million people living with HIV/AIDS worldwide are co-infected with tuberculosis (TB) [1]. Rifampicin is the most powerful component of current TB treatment, but is also a strong inducer of cytochrome P450 enzymes, resulting in drug-drug interactions with antiretroviral therapy [2, 3], in particular non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs).

The World Health Organization (WHO) recommends to combine rifampicin-based TB treatment with efavirenz-based antiretroviral therapy in TB-HIV co-infected patients [3]. The modest decrease in concentrations of the NNRTI efavirenz is not expected to be clinically relevant [4, 5]. Although rifampicin reduces concentrations of the NNRTI nevirapine by 20–55% [6], collected experience is sufficient to make nevirapine an alternative to efavirenz among non-Caucasian patients receiving rifampicin [7, 8]. A disadvantage of NNRTI drugs is the low genetic barrier for the development of resistance; only one point mutation is required to confer high-level cross-class resistance [9].

One alternative for patients with NNRTI-resistant HIV or those unable to take NNRTIs would be triple nucleoside regimens without interaction to rifampicin. However, these regimens are associated with less antiretroviral activity [10] and therefore not advisable. An equally active alternative to NNRTI-based therapy among patients being treated with rifampicin may be PI-based antiretroviral therapy. However, standard PI doses, whether boosted with ritonavir or not, cannot be given with rifampicin because of large decreases in PI concentrations [11–14].

In HIV infected adults without active tuberculosis, WHO recommends lopinavir/ritonavir at 400/100 mg twice daily (BID) in combination with two nucleosides as PI-based therapy [15]. We have previously evaluated the pharmacokinetics of two adjusted dose regimens of lopinavir/ritonavir (800/200 mg BID and 400/400 mg BID) soft gel capsules when combined with rifampicin in healthy volunteers [11]. Because of more variable pharmacokinetics of lopinavir in the better tolerable arm of 800/200 mg lopinavir/ritonavir, therapeutic drug monitoring (TDM) appeared to be indicated. In the absence of TDM, 400/400 mg lopinavir/ritonavir could be combined with rifampicin, while closely monitoring for liver enzyme elevations.

Recently, a new tablet formulation of lopinavir/ritonavir has become available. Compared to the soft gel capsule, the novel tablet formulation shows reduced pharmacokinetic variability and exhibits slightly higher (18%) bioavailability, irrespective of concomitant food intake [16].

The aim of this study was to evaluate the pharmacokinetics of two adjusted dose regimens of lopinavir/ritonavir tablets in combination with rifampicin in healthy volunteers. Due to an unexpectedly high incidence of adverse events, this study was terminated prematurely.

## Methods

### Study design

The study was designed as an open-label, sequential, 2-period, phase IV multiple dose trial in healthy volunteers. From day 1 until day 5, subjects received 600 mg rifampicin once daily (QD). From day 6 until day 15, subjects were randomized to receive either three (600/150 mg) or four (800/200 mg) of the new lopinavir/ritonavir (Kaletra®) tablets BID, combined with 600 mg rifampicin QD. On days 1, 3, 5, 6, 7, 9, 11, 13 and 15 study medication was planned to be taken under observation and immediately after a standardized breakfast ( $\pm$  350 kcal,  $\pm$  13 g. of fat). The other drug doses were taken at home. All subjects gave written informed consent and the Ethical Review Board of Radboud University Nijmegen Medical Centre, The Netherlands, approved the study.

### Subjects

Healthy subjects, aged 18-55 years were eligible for enrolment after pre-entry and laboratory evaluation. The main exclusion criteria were: positive HIV test result; positive hepatitis B or C test result; positive Mantoux test result; abnormal clinical laboratory test results and abnormal ECG.

### Pharmacokinetic assessment

Blood samples of 5 ml were planned to be collected at the following time points; on day 5 just before and two hours post-dose ( $C_{2h}$ ) for the determination of rifampicin (and desacetyl rifampicin) plasma concentrations; on day 7, 9, 11 and 13 pre-dose

for the determination of plasma concentrations of lopinavir and ritonavir; and on day 15 pre-dose until 12 hours post-dose for the analysis of all three drugs. Blood samples were stored in the refrigerator immediately and centrifuged at 2,500 g for 10 minutes. Plasma was separated and stored below -40°C (for the determination of lopinavir and ritonavir) or -80°C (for the determination of rifampicin and desacetyl rifampicin) within 4 hours after collection. All procedures concerning collection and storage of drug samples have been validated.

### Bioanalysis

Plasma lopinavir, ritonavir, rifampicin and desacetyl rifampicin concentrations were determined by validated high-performance liquid chromatography (HPLC) methods [17, 18].

### Safety and tolerability

We planned to perform clinical chemistry and hematology tests and to ask subjects about the occurrence of adverse events at each study visit on day 1, 3, 5, 6, 7, 9, 11, 13 and 15. Adverse events, clinical chemistry and hematology test results were graded according to the grading system of the Division of AIDS [19]. After termination of the study, hepatitis B and C tests were repeated. In addition, Epstein-Barr virus and cytomegalovirus antibody tests were performed to exclude acute viral infection.

## Results

### Subjects

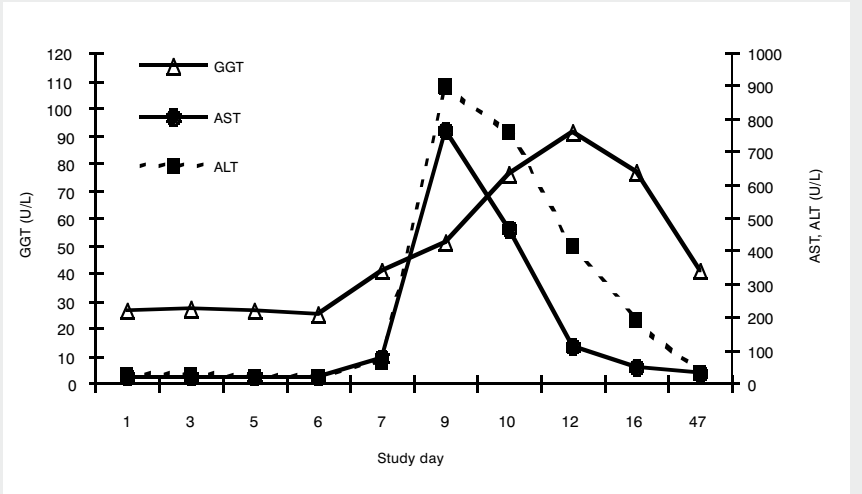
Forty healthy volunteers were enrolled in the study. The first group consisted of eleven subjects of whom eight were Caucasians, two were Caucasian-black and one was black. The median (range) age, body weight and body mass index were 24 (19-51) years, 63 (53-92) kg and 22 (19-30) kg/m<sup>2</sup>, respectively. Five subjects (three females) received 600/150 mg lopinavir/ritonavir BID and six (four females) received 800/200 mg lopinavir/ritonavir BID in addition to rifampicin 600 mg. The remaining 29 subjects were withdrawn from the study after receiving only one dose of rifampicin (n=10) or no study medication at all (n=19).

**Table 1** Distribution of adverse events (n, %) in eleven healthy volunteers. Medication was stopped on day 7 or 8.

Adverse event	Day 1-11 (overall)	Day 1-5 (rifampicin)	Day 6- 11 (day 6-7/8: rifampicin and lopinavir/ritonavir)
Flatulence	1 (9%)	0	1 (9%)
Headache	1 (9%)	1 (9%)	0
Rash			
Grade 1	1 (9%)	1 (9%)	0
Fever			
Grade 1	1 (9%)	0	1 (9%)
Myalgia			
Grade 2	1 (9%)	0	1 (9%)
Joint pain			
Grade 1	2 (18%)	2 (18%)	0
Fatigue			
Grade 1	1 (9%)	0	1 (9%)
Grade 2	1 (9%)	0	1 (9%)
Trismus*	1 (9%)	0	1 (9%)
Diarrhoea			
Grade 1	2 (18%)	1 (9%)	2 (18%)
Grade 2	1 (9%)	0	1 (9%)
Nausea			
Grade 1	3 (18%)	1 (9%)	2 (18%)
Grade 2	8 (73%)	2 (18%)	7 (64%)
Vomiting			
Grade 2	3 (27%)	0	3 (27%)
Grade 3	6 (55%)	0	6 (55%)
Pruritis			
Grade 1	3 (27%)	3 (27%)	3 (27%)
Grade 2	1 (9%)	1 (9%)	0
Headache	1 (9%)	1 (9%)	0
ALT			
Grade 2	2 (18%)	0	2 (18%)
Grade 3	1 (9%)	0	1 (9%)
Grade 4	8 (73%)	0	8 (73%)
AST			
Grade 2	2 (18%)	0	2 (18%)
Grade 3	1 (9%)	0	1 (9%)
Grade 4	8 (73%)	0	8 (73%)
GGT			
Grade 1	4 (36%)	0	4 (36%)
Grade 2	2 (18%)	0	2 (18%)
Grade 3	1 (9%)	0	1 (9%)
Bilirubin			
Grade 2	3 (27%)	0	3 (27%)
Grade 3	5 (45%)	0	5 (45%)

\* Probably related to excessive metoclopramide usage.  
ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT (gamma glutamyl transferase).

**Figure 1** Mean clinical parameters versus time in eleven healthy volunteers until study day 47 (day 1-5: rifampicin only; day 6-7/8: rifampicin and lopinavir/ritonavir; day 7/8-47: no medication).



GGT (gamma glutamyl transferase), AST (aspartate aminotransferase), ALT (alanine aminotransferase).

### Pharmacokinetics

The geometric mean (range) of rifampicin and desacetyl-rifampicin  $C_{2h}$  on day 5 (rifampicin only) was 11.3 (6.8-17.4) and 1.12 (0.74-2.37) mg/L, respectively (n=11). In five subjects, plasma concentrations of lopinavir and ritonavir were undetectable in the morning of day 7 due to vomiting after the evening dose on day 6. In the remaining six subjects, the geometric mean (range) of the lopinavir and ritonavir trough concentrations on day 7 were 7.9 (6.6-10.3) mg/L and 0.71 (0.35-1.61) mg/L in the 600/150 mg group (n=3) and 10.9 (8.3-13.8) mg/L and 1.20 (0.64-1.85) mg/L in the 800/200 mg group (n=3). The median (range) of the rifampicin and desacetyl-rifampicin trough concentrations in the morning of day 7 were 0.41 (0.0-1.13) mg/L and 0.40 (0.0-0.83) mg/L in the 600/150 mg group (n=5) and 0.47 (0.0-1.17) mg/L and 0.21 (0.0-1.12) mg/L in the 800/200 mg group (n=6).

### Tolerability

No major complaints or abnormal AST/ALT levels occurred during day 1–5, when rifampicin was given only (Table 1). After addition of lopinavir/ritonavir, eight

subjects suffered from both nausea and vomiting, one had nausea only, and one vomited only. Eight subjects were given the antiemetic drug metoclopramide. All six subjects in the 800/200 mg lopinavir/ritonavir group vomited, compared to three out of five in the 600/150 mg group. On day 7, increases in AST and ALT levels were reported in all subjects and increases in GGT in all but one subject. Study medication was stopped on day 7 for 8 subjects and on day 8 for the remaining three subjects. The AST/ALT levels continued to rise and peaked (range 125-1400 and 201-1657 U/L, respectively) on days 9-10 (Figure 1). The GGT levels increased more gradually and peaked (range 21-177 U/L) on days 10-12 (Figure 1). Four out of six subjects in the 800/200 mg lopinavir/ritonavir group experienced grade 4 AST or ALT toxicity versus four out of five in the 600/150 mg group. All clinical parameters returned to normal within six weeks after study termination (Figure 1). Test results for hepatitis B and C, Epstein-Barr virus and cytomegalovirus were all negative after study termination.

## Discussion

This study showed an unexpected high incidence of nausea, vomiting and liver enzyme elevations when a higher than standard dose of the recently introduced lopinavir/ritonavir tablets was combined with rifampicin in healthy volunteers. Therefore, the study was terminated prematurely.

In our previous study in which rifampicin and adjusted doses of lopinavir/ritonavir capsules were combined [11], toxicity of this magnitude was not observed. However, the current study differed from our previous study [11] in several aspects, and this may explain the higher incidence of adverse events. Firstly, rifampicin was introduced prior to lopinavir/ritonavir; secondly, the adjusted dose of lopinavir/ritonavir tablets was not escalated; and thirdly, the new lopinavir/ritonavir tablet formulation was used instead of the capsule formulation.

Firstly, the sequence of introduction of rifampicin and lopinavir/ritonavir may have played a role. Termination of a study because of unexpected hepatotoxicity in healthy volunteers using the combination of rifampicin and saquinavir/ritonavir, was reported before [20]. The majority of the adverse events was observed in the study

arm in which rifampicin usage was followed by the combination of rifampicin and the PI [20], analogous to the current study. However, this sequence is a reflection of the clinical situation of TB-HIV co-infected patients as TB is preferably treated first. Besides, TB-HIV co-infected patients seem to tolerate the combination of rifampicin and saquinavir/ritonavir well in both sequences [14, 21], suggesting a yet unidentified difference between patients and healthy volunteers. It has not been investigated so far, whether TB-HIV co-infected patients tolerate the combination of rifampicin and lopinavir/ritonavir better than healthy volunteers.

With respect to the second difference, the absence of a dose escalation scheme for the adjusted dose of lopinavir/ritonavir may have contributed to the high incidence of adverse events in the current study. We decided to omit dose escalation because the liver enzymes had been induced by rifampicin already. The efflux transporter P-glycoprotein (P-gp) can mediate the transport of lopinavir and ritonavir [22, 23] as well as rifampicin [24]. A time-dependent effect by lopinavir/ritonavir on P-gp was previously suggested; inhibition after single-dose and induction after repeated administration [25]. The immediate administration of the high dose of lopinavir/ritonavir in the current study may have resulted in inhibition of the efflux transporter P-gp and therefore to the relatively high trough concentrations of the study medication observed. This may explain part of the adverse events.

Finally, unidentified toxic excipients of the new tablet formulation and its slightly higher bioavailability may have played a role. Studies show that this slight increase in bioavailability of lopinavir/ritonavir tablets may compensate for the inductive effect of efavirenz [26]. This same compensation may occur when combined with rifampicin, as in the current study in which we found relatively high non steady-state lopinavir trough levels compared to the steady-state levels in the previous study [11]. In short, data from interaction studies with one formulation cannot be extrapolated to another formulation.

In conclusion, this study demonstrates the challenging character of the compatibility of rifampicin and PIs in TB-HIV therapy. We advise that further studies investigating the interaction between rifampicin and lopinavir/ritonavir tablets be performed in the actual target population of TB-HIV co-infected patients rather than in healthy volunteers. Awaiting these results, simultaneous treatment with rifampicin and



lopinavir/ritonavir should not be applied. In case no alternatives are available, extreme caution and close monitoring of liver enzymes and plasma drug concentrations is warranted.

## Acknowledgements


We would like to thank the healthy volunteers for their participation in this study. The staff at the Clinical Research Centre Nijmegen (CRCN), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands is acknowledged for their cooperation and effort. The technicians of the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands are acknowledged for the analysis of the plasma samples. This study was funded by Radboud University Nijmegen Medical Centre.

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Rafaëlla L'homme<sup>1,2</sup>, Hanneke Nijland<sup>1,2</sup>, Luuk Gras<sup>3</sup>, Rob Aarnoutse<sup>1,2</sup>, Reinout van Crevel<sup>2,4</sup>, Martin Boeree<sup>2,5</sup>, Kees Brinkman<sup>6</sup>, Jan Prins<sup>7</sup>, Job Juttmann<sup>8</sup>, David Burger<sup>1,2</sup>

<sup>1</sup> Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>2</sup> Nijmegen University Centre for Infectious diseases (NUCI), Nijmegen, The Netherlands; <sup>3</sup> HIV Monitoring Foundation, Amsterdam, The Netherlands; <sup>4</sup> Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; <sup>5</sup> Department of Pulmonary Diseases, Radboud University Nijmegen Medical Centre and University Lung Centre Dekkerswald, Nijmegen, The Netherlands; <sup>6</sup> Department of Internal Medicine, Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands; <sup>7</sup> Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, Amsterdam, The Netherlands; <sup>8</sup> Department of Internal Medicine, St Elisabeth Hospital, Tilburg, The Netherlands

*Submitted*

## Chapter 8

Clinical experience with  
the combined use of lopinavir/  
ritonavir and rifampicin

## Abstract

**Objective:** A retrospective study was conducted to describe the clinical experience in the Netherlands with the combined use of lopinavir/ritonavir and rifampicin.

**Methods:** Adult HIV-infected patients treated concomitantly with lopinavir/ritonavir and rifampicin were selected from the Dutch ATHENA cohort and evaluated with regard to 6 items: lopinavir/ritonavir dose and formulation, sequence of drug administration, lopinavir trough plasma concentration, plasma viral load and tolerability.

**Results:** Thirty-four patients with a median age of 36 years were included. Overall, only 15% used the recommended increased dose of lopinavir/ritonavir. Of the 23 patients on a non-adjusted dose of lopinavir/ritonavir, 4 out of 6 (67%) had a subtherapeutic lopinavir trough plasma concentration and 3 out of 8 (38%) had a detectable viral load after at least 4 months of concomitant treatment. Two out of 5 (40%) patients on a recommended increased dose of lopinavir/ritonavir prematurely stopped the drug combination because of adverse events vs. 4 out of 23 (17%) on a non-adjusted dose and 1 out of 6 (17%) on a slightly increased dose.

**Conclusions:** Combined use of lopinavir/ritonavir and rifampicin is challenging as it implies balancing between suboptimal efficacy and toxicity. An adequately powered prospective study in HIV-infected patients is needed to investigate the consequences of inferior exposure to lopinavir due to non-adjusted doses of lopinavir/ritonavir versus the tolerability of recommended increased doses.

## Introduction

Tuberculosis (TB) is the most prevalent opportunistic infection among HIV-infected patients worldwide [1]. Rifampicin is an important drug in the treatment of TB and has strong liver enzyme inducing capacity, resulting in drug-drug interactions with antiretroviral therapy [2].

Rifampicin-based TB treatment can be combined with antiretroviral therapy based on the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz [1], since the decrease in exposure to efavirenz due to co-administration of rifampicin is not expected to be clinically relevant [3, 4]. However, efavirenz cannot be used in case of pregnancy, intolerance or resistance to the drug [5]. The NNRTI nevirapine is considered to be an alternative to efavirenz among non-Caucasian patients receiving rifampicin [6, 7], despite the fact that rifampicin reduces the exposure to nevirapine by 20-55% [8].

An alternative for patients with NNRTI-resistant HIV who are treated with rifampicin would be protease inhibitor (PI)-based antiretroviral therapy [1]. However, rifampicin has been shown to cause large decreases in exposure to PIs [9-12]. We have previously performed two studies in healthy volunteers to evaluate whether adjustment of the standard lopinavir/ritonavir dose of 400/100 mg twice daily (BID) could compensate for the drug interaction with rifampicin [9, 13].

Our first study demonstrated that, on average, rifampicin and increased doses of lopinavir/ritonavir soft gel capsules (SGCs; 800/200 mg BID and 400/400 mg BID) resulted in adequate exposure to lopinavir in healthy volunteers [9]. However, therapeutic drug monitoring (TDM) was advised when combining rifampicin with 800/200 mg lopinavir/ritonavir BID and close monitoring for liver enzyme elevations was advocated when combined with 400/400 mg BID.

A new tablet formulation of lopinavir/ritonavir became available in 2006 to replace the former SGC formulation. In our second study, we aimed to evaluate the pharmacokinetics of two adjusted dose regimens of lopinavir/ritonavir tablets (600/150 mg BID and 800/200 mg BID) in combination with rifampicin in healthy volunteers [13]. However, the study was terminated prematurely due to an unexpectedly high incidence of nausea, vomiting and liver enzyme elevations.

In the absence of similar interaction studies in patients, we performed a retrospective analysis of observational data from the ATHENA cohort [14] to describe the clinical experience in the Netherlands of combining lopinavir/ritonavir and rifampicin.

## Methods

Adult ( $\geq 18$  years) HIV-infected patients treated concomitantly with lopinavir/ritonavir and rifampicin were selected from the Dutch ATHENA cohort [14]. Baseline was defined as the 6 months period before the start of concomitant treatment. Patient demographic and clinical characteristics were collected at baseline. During the period of concomitant use, the patients were evaluated with regard to six items: lopinavir/ritonavir dose and formulation, sequence of drug administration, lopinavir trough plasma concentration (i.e. the concentration just prior to the next dose), plasma viral load, and tolerability.

Recommendations of lopinavir/ritonavir dose increment to 400/400 or 800/200 mg BID when combined with rifampicin [1, 15] are based on a pharmacokinetic interaction study in healthy volunteers presented at the end of September 2002 [16]. In the current analysis, prescribed doses of lopinavir/ritonavir in combination with rifampicin were classified as non-adjusted (400/100 mg BID or 800/200 mg once daily (QD)), slightly adjusted (500/125 mg as liquid or 533/133 mg as SGC, both BID) or adjusted according to recommendation (400/400 or 800/200 mg BID). The overall proportions of prescribed lopinavir/ritonavir formulations (SGC, tablet, liquid) and sequences of drug administration (rifampicin lead-in, lopinavir/ritonavir lead-in, simultaneous initiation) were determined. We determined the overall proportion of lopinavir/ritonavir dose adjustments when combined with rifampicin and the proportion before and after the recommendation of dose adjustment was first presented (October 1, 2002) [16].

Lopinavir trough plasma concentrations were classified as subtherapeutic ( $<1.0$  mg/L) or therapeutic ( $\geq 1.0$  mg/L) [17]. The proportion of patients with a subtherapeutic lopinavir trough plasma concentration was determined in those on a non-adjusted, slightly adjusted or recommended dose of lopinavir/ritonavir.

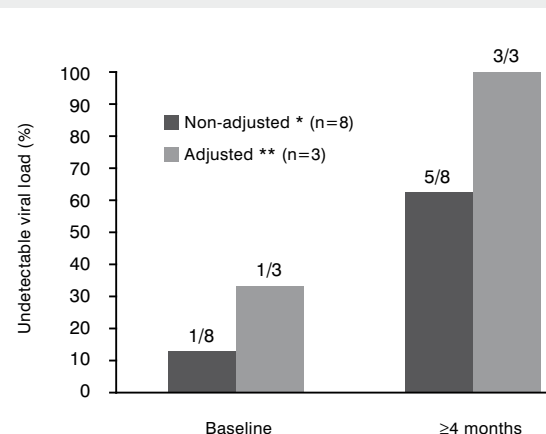
For patients who used the drug combination of lopinavir/ritonavir and rifampicin for at least 4 months, plasma viral load data were collected until the end of concomitant treatment. Viral loads were classified as undetectable (HIV-RNA  $<50$  copies/ml) or detectable ( $\geq 50$  copies/ml). The proportion of patients with an undetectable plasma viral load at baseline and at the end of concomitant treatment was determined in those on a non-adjusted, slightly adjusted or recommended dose of lopinavir/ritonavir.

Acute, premature termination of lopinavir/ritonavir and rifampicin was defined as stopping concomitant treatment within 4 weeks from the start. Reported adverse events and grade of liver toxicity expressed by elevation of aspartate and alanine aminotransferase (AST/ALT) were collected for patients who prematurely stopped and for those who continued the drug combination [18]. The clinicians of patients who prematurely terminated the drug combination were asked to review the patient file and report the reason for stopping. We calculated the proportion of patients with premature stops because of adverse events overall and according to the 3 dose groups. In addition, we identified the formulations and lead-in drugs that were used by the patients who prematurely stopped the drug combination because of adverse events.

## Results

The 34 patients (8 women, 26 men) who were included in the study originated from Sub-Saharan Africa ( $n=12$ ), The Netherlands ( $n=8$ ), Latin America ( $n=7$ ), The Netherlands Antilles ( $n=3$ ), South East Asia ( $n=2$ ) or Middle East / North Africa ( $n=2$ ). Combined treatment with lopinavir/ritonavir and rifampicin was initiated between October 2000 and February 2007. 32 Patients (94%) were treated for TB. Antiretroviral co-medication consisted of zidovudine and lamivudine ( $n=12$ ), stavudine and lamivudine ( $n=4$ ), lamivudine and tenofovir ( $n=4$ ), zidovudine, lamivudine and abacavir ( $n=4$ ) or alternative combinations ( $n=10$ ). Median (interquartile range) age, weight, CD4 cell count and plasma viral load at baseline were 36 (32, 43) years, 62 (55, 70) kg, 105 (27, 248) cells/ $\mu$ l and 75,750 ( $<50$ , 204,085) copies HIV-RNA/ml.

**Figure 1** Proportion of patients with an undetectable viral load according to lopinavir/ritonavir dose group at baseline and after at least 4 months of co-administration with rifampicin.



\* 400/100 mg lopinavir/ritonavir twice daily

\*\* 533/133 mg (n=2) or 800/200 mg (n=1) lopinavir/ritonavir twice daily

Overall, 23 of 34 (68%) patients were on a non-adjusted dose of lopinavir/ritonavir (400/100 mg BID or 800/200 mg QD), 6 (18%) on a slightly adjusted dose (500/125 or 533/133 mg BID) and 5 (15%) on a recommended dose (400/400 or 800/200 mg BID). 8 Patients started concomitant treatment before the recommendation of lopinavir/ritonavir dose adjustment was first presented; 26 patients started concomitant treatment after the recommendation of lopinavir/ritonavir dose adjustment became available in the public domain [16]. Of the 8 patients who started concomitant treatment before the recommendation of lopinavir/ritonavir dose adjustment was first presented, 7 (88%) were on a non-adjusted dose, 1 (13%) on a slightly adjusted dose and none on a recommended dose. Of the 26 patients who started concomitant treatment after the recommendation of lopinavir/ritonavir dose adjustment was in the public domain, 16 (62%) were on a non-adjusted dose, 5 (19%) on a slightly adjusted dose and 5 (19%) on a recommended dose.

Lopinavir trough plasma concentrations during combined use were available for 6 of 23 (26%) patients on a non-adjusted dose of lopinavir/ritonavir, none of 6 patients on a

slightly adjusted dose and 2 of 5 (40%) patients on a recommended dose. Four of 6 (67%) patients on a non-adjusted dose of lopinavir/ritonavir had a subtherapeutic lopinavir trough plasma concentration, whereas the 2 patients on a recommended dose had a therapeutic concentration.

Viral load data were available for all 11 patients who used lopinavir/ritonavir and rifampicin concomitantly for at least 4 months. In Figure 1, the proportion of patients with an undetectable plasma viral load at baseline and at the end of concomitant treatment is shown according to lopinavir/ritonavir dose group.

In Table 1, the reported adverse events and toxicity grade of AST/ALT elevations are shown for the 12 (35%) patients who prematurely stopped concomitant lopinavir/ritonavir and rifampicin treatment within 4 weeks and for the 22 (65%) who continued the drug combination. Nausea and malaise were the most experienced adverse events, but were only reported in the group that prematurely discontinued the drug combination. Grade 2 AST/ALT elevations were more often reported in the group that continued the drug combination. Overall, 7 of 34 (21%) patients prematurely stopped the combination within 4 weeks because of acute adverse events; this was 4 of 23 (17%) on a non-adjusted dose, 1 of 6 (17%) on a slightly adjusted dose and 2 of 5 (40%) on a recommended dose of lopinavir/ritonavir (Table 2).

Twenty-eight of 34 (82%) patients were on the SGC formulation, 4 (12%) on the tablet formulation and 2 (6%) on the liquid formulation of lopinavir/ritonavir. Twenty-three (68%) patients had a rifampicin lead-in, 10 (29%) had a lopinavir/ritonavir lead-in and 1 (3%) initiated both drugs simultaneously. The formulations and lead-in drugs that were used by the 7 patients who prematurely stopped the drug combination because of adverse events are shown in Table 2; none of them was using tablets and the proportion of lead-in drugs was comparable to the overall proportion.

## Discussion

This retrospective study demonstrates that the dose of lopinavir/ritonavir was usually not increased when combined with rifampicin in Dutch HIV-infected patients. This may have led to the relatively high proportion of subtherapeutic lopinavir trough

**Table 1** Distribution of adverse events and grade of AST<sup>a</sup>/ALT<sup>b</sup> in those who prematurely terminated (<4 weeks) and those who continued (≥ 4 weeks) co-administration of lopinavir/ritonavir and rifampicin.

Category	Adverse event and grade of AST <sup>a</sup> /ALT <sup>b</sup>	<4 weeks n=12	≥4 weeks n=22
Cardiovascular	Sinus tachycardia		1 (5%)
Chemistries	Slightly elevated LDH <sup>c</sup> /ALP <sup>d</sup>	1 (8%)	
Dermatological	Rash		2 (9%)
Gastrointestinal	Nausea	4 (33%)	
	Diarrhoea	2 (17%)	1 (5%)
	Bad taste mouth	1 (8%)	
	Vomiting	1 (8%)	
Genitourinary	Renal disorder		1 (5%)
Infection	Candidiasis genital		1 (5%)
	Cerebral toxoplasmosis		1 (5%)
	Conjunctivitis		1 (5%)
	Herpes zoster		1 (5%)
Metabolic	Diabetes Mellitus		1 (5%)
Neurologic	Paraesthesia	1 (8%)	1 (5%)
	Peripheral neuropathy		1 (5%)
	Bad dreams		1 (5%)
Systemic	Malaise	3 (25%)	
	Fatigue	1 (8%)	1 (5%)
	Fever	1 (8%)	1 (5%)
	Cachexia	1 (8%)	
	Liver toxicity	1 (8%)	
	Pain upper abdomen	1 (8%)	
	AST <sup>a</sup> / ALT <sup>b</sup>		
	Grade 0	2 (17%)	1 (5%)
	Grade 1		1 (5%)
	Grade 2		5 (23%)
	Grade 3	1 (8%)	2 (9%)

Values given are n (%)

<sup>a</sup> aspartate aminotransferase<sup>b</sup> alanine aminotransferase<sup>c</sup> lactate dehydrogenase<sup>d</sup> alkaline phosphatase**Table 2** Adverse events responsible for premature termination of concomitant treatment with lopinavir/ritonavir and rifampicin in 7 out of 34 patients.

Patient	LPV/rtv <sup>a</sup> dose	Adverse event	LPV/rtv <sup>a</sup> Formulation	Lead-in drug
1	Non-adjusted <sup>b</sup>	Liver toxicity, malaise, nausea, vomiting	SGC <sup>h</sup>	Rifampicin
2		Nausea		
3		Diarrhoea		LPV/rtv <sup>a</sup>
4	Slightly adjusted <sup>c</sup>	Immune reconstitution syndrome		Rifampicin
5		Pain upper abdomen		
6	Recommended <sup>d</sup>	Bad taste mouth, diarrhoea, nausea, paraesthesia	liquid	LPV/rtv <sup>a</sup>
7		Nausea, slightly elevated LDH <sup>e</sup> , ALP <sup>f</sup> and ALT <sup>g</sup>		None

<sup>a</sup> lopinavir/ritonavir<sup>b</sup> 400/100 mg twice daily<sup>c</sup> 533/133 mg twice daily<sup>d</sup> 400/400 mg (n=1) or 800/200 mg (n=1) twice daily<sup>e</sup> lactate dehydrogenase<sup>f</sup> alkaline phosphatase<sup>g</sup> alanine aminotransferase<sup>h</sup> soft gel capsule

plasma concentrations and detectable viral loads. The low adherence to this particular dose recommendation is in line with a recent study demonstrating that a minority of patients received dose adjustments to compensate for interactions between antiretroviral therapy and rifabutin [19].

Recommendations of lopinavir/ritonavir dose increment when combined with rifampicin [1, 15] have been based on one of our previous pharmacokinetic interaction studies [16]. Although the uptake of this dose recommendation was noticed to some extent after presentation of the particular study [16], general adherence to the recommendation was low. This may be due to clinicians not being aware of the recommendation at the time of prescription, because of slow integration into guidelines [1, 15] and medication monitoring systems. Another reason may be that clinicians did not follow this recommendation as it was only based on a pharmacokinetic interaction study in healthy volunteers [9] without evidence based confirmation of clinical relevance and tolerability of dose increments in HIV-infected patients.



One would expect that lopinavir plasma concentrations are monitored when rifampicin is combined with a non-adjusted dose of lopinavir/ritonavir, but in our study plasma concentrations were monitored in a quarter of such cases only. This is a concern, as two-thirds of the patients on a non-adjusted lopinavir/ritonavir dose appeared to have subtherapeutic lopinavir trough plasma concentrations [17] in accordance with one of our previous studies in healthy volunteers [9]. Moreover, none of the doses that resulted in subtherapeutic lopinavir trough plasma concentrations were adjusted.

Viral loads have been monitored in all patients in our cohort who were concomitantly treated with rifampicin and lopinavir/ritonavir for at least 4 months. Unfortunately, the number of participants with this duration of concurrent treatment was too small to draw firm conclusions. Whereas the available data suggest that inappropriate virological response may be explained by low lopinavir exposure due to non-adjusted doses, alternative factors, such as non-adherence to antiretroviral therapy or insensitivity of the virus, may also have contributed. Also, a difference between these groups in the proportion of patients with an undetectable viral load at baseline (1/8 vs. 1/3) cannot be ruled out.

The occurrence of nausea, vomiting and liver enzyme elevations when combining lopinavir/ritonavir and rifampicin in our cohort of HIV-infected patients was far less than previously reported in healthy volunteers on recommended increased doses of the SGC formulation [9] and in particular the tablet formulation [13] of lopinavir/ritonavir. The observed difference in hepatotoxicity is striking considering the fact that 94% of the HIV-infected patients were treated with other tuberculostatic agents in addition to rifampicin, compared to none of the healthy volunteers. The overall better tolerability of the drug combination in the current study compared to our previous studies [9, 13] may be explained by differences in drug dose, study population, drug formulation and sequence of drug administration.

Firstly, the fact that the lopinavir/ritonavir dose was increased as recommended in only the minority of patients on rifampicin may have contributed to the lower incidence of adverse events in the current study. This explanation is supported by our observation that a higher proportion of those on a recommended lopinavir/ritonavir dose prematurely stopped the drug combination because of adverse events.

Secondly, the fact that the current study was performed in HIV-infected patients rather than healthy volunteers may have led to the better tolerability of the combination lopinavir/ritonavir and rifampicin. This explanation is supported by other studies demonstrating that HIV patients tolerate another PI-combination, saquinavir/ritonavir, and rifampicin better than healthy volunteers [12, 20, 21].

Although the magnitude of toxicity in our previous studies in healthy volunteers was more severe when using tablets [13] rather than SGCs [9], in the present study we did not find any support that this may be caused by the formulation itself. In another study, without rifampicin, it is even claimed that the new tablet formulation of lopinavir/ritonavir is better tolerated than the SGC [22].

Previous studies in healthy volunteers suggest that the rifampicin lead-in before the introduction of protease inhibitors may have contributed to the high incidence of toxicity observed [13, 20, 23]. However, we could not confirm that the sequence of drug administration may have led to the overall better tolerability in HIV-infected patients.

Several limitations of our study need to be mentioned. This was a retrospective analysis of an observational cohort. Data were not collected on prespecified time points and were not always available. However, the quality of the data in the ATHENA cohort is independently monitored [14]. In case of missing or possible inaccuracy of data, we contacted the treating physician for clarification. As the design of the study was observational and not a randomized study, observed findings may have been confounded. Also, the number of patients involved in this evaluation was limited. Therefore, statistical analyses were not performed.

In conclusion, a small minority of HIV-infected patients in the Netherlands has been treated with the recommended increased dose of lopinavir/ritonavir in combination with rifampicin. This resulted in inferior exposure to lopinavir and possibly related virological failure. The overall tolerability of the drug combination was better than previously reported in healthy volunteers, whereas a higher proportion of those on a recommended increased dose prematurely stopped the combination because of adverse events. Concomitant use of lopinavir/ritonavir and rifampicin remains challenging. An adequately powered prospective study in HIV-infected patients is



needed to investigate the consequences of inferior exposure to lopinavir due to non-adjusted lopinavir/ritonavir doses versus the tolerability of recommended increased doses.

## Acknowledgements

We would like to thank all physicians for providing additional clinical information.

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**General discussion**

## Introduction

This thesis presents the first output of a North-South collaboration in clinical pharmacological research of HIV treatment in which the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre was involved. Sub-Saharan Africa continues to be the region most affected by HIV. Although the access to adult HIV treatment in resource-poor countries is being improved and an increasing amount of data is generated on treatment efficacy, limited data are available on clinical pharmacology. In this thesis, a collaboration between partners from The Netherlands, United Kingdom, Zambia and Tanzania has led to clinical pharmacological research of HIV treatment that is specifically relevant for resource-limited countries. **Part I** deals with the optimization of paediatric regimens for the scale-up of HIV treatment in resource-limited countries. **Part II** focuses on therapeutic drug monitoring in HIV-infected adults and the optimization of a cost-effective strategy for the reduction of mother-to-child transmission in low-income countries. **Part III** describes the challenges of combined treatment with lopinavir/ritonavir and rifampicin for HIV-TB co-infection. Overlooking the presented studies, five topics that are relevant for developing countries were selected for discussion: (1) access to HIV treatment; (2) monitoring of HIV treatment; (3) prevention of mother-to-child transmission of HIV; (4) treatment of HIV-TB co-infection; and (5) North-South collaboration in clinical pharmacological research. This general discussion is concluded with future perspectives.

### 1. Access to HIV treatment

The recent improvement in the access to HIV treatment in developing countries has largely depended on the introduction of cheap generic fixed dose combination (FDC) tablets with standard doses of antiretroviral drugs for adults. The lack of affordable and appropriate paediatric antiretroviral drug formulations has led to prescription of generic adult FDC tablets to children. Several reasons may have contributed to the delay in development of generic paediatric formulations for HIV-infected children in developing countries.

Firstly, the incorrect assumption that there is no need of a generic paediatric formulation may have hindered its development. HIV-infected children cannot simply be regarded as small adults, pharmacologically speaking (**Chapter 1**).

For example, children metabolize the antiretroviral drug nevirapine more rapidly than adults. **Chapter 2** demonstrates that it is impossible to achieve adequate nevirapine concentrations without risking overdosing with stavudine in HIV-infected children receiving divided adult FDC tablets (Triomune; Cipla Ltd., India; 200mg nevirapine, 30 or 40 mg stavudine, 150 mg lamivudine). This is of concern, as children with subtherapeutic nevirapine concentrations are at risk of virological failure and development of resistance [1, 2].

Secondly, the incorrect assumption that a generic paediatric FDC formulation should be liquid may have tempered the enthusiasm for its development by generic manufacturers. The development of liquid formulations can be complex due to problems with stability, solubility and taste. For example, no liquid formulations have been marketed for the insoluble drugs indinavir and saquinavir, and the liquid formulations of efavirenz, ritonavir and lopinavir/ritonavir all have an unpleasant taste. The current development of a lopinavir/ritonavir tablet with half the strength of the adult formulation will offer an attractive alternative to older children in the near future. The time and energy spent trying to develop liquid paediatric FDC formulations is better used in the manufacture of small, dispersible, crushable and scored FDC tablets, which are easier to develop, dispense, transport, store and administer [3]. The development of separate tablet formulations for young and older children would minimize the number of tablets to be taken per dosing interval. On the other hand, management of stocks would be facilitated by the introduction of only one paediatric formulation.

Thirdly, the fact that generic paediatric FDC formulations may be less lucrative for commercial institutions could also explain the delay in development. Only 2.5 million children out of 33.5 million people were estimated to be living with HIV at the end of 2007 [4], indicating a relatively small market for generic paediatric FDC formulations. In addition, developed countries that can afford to pay a higher price for paediatric formulations may not be as interested in generic paediatric FDC tablets as resource-poor countries.

Finally, the fact that global efforts such as the “3 by 5” initiative of the World Health Organization (WHO) have mainly focussed on the access to HIV treatment for adults [5] may have contributed to the delay in development of generic paediatric formulations.

It was only recently that a WHO paediatric antiretroviral working group (PAWG) started to encourage the development of paediatric-friendly FDC tablets and the universal access to safe treatment for HIV-infected children [6]. PAWG identified a prioritized list of paediatric products and used a generic dosing tool, assessing the intended dose delivered, to arrive at recommended doses. Weight-band based dosing tables were developed to simplify dosing of antiretroviral drugs in resource-limited settings. For drugs that should be dosed by body surface area, such as nevirapine, doses were converted into weight bands. PAWG advised a higher nevirapine to stavudine and lamivudine dose ratio for the paediatric version of the adult FDC Triomune investigated in **Chapter 2**. In addition to the initiatives of WHO [6], the new European paediatric regulation has led to an obligation for paediatric research for every new drug developed for adults and having a potential use for children, in exchange for a six month extension of the supplementary protection certificate.

In response to the urgent need for generic paediatric FDC tablets, Cipla Ltd. has developed Triomune Baby and Junior with higher nevirapine to stavudine and lamivudine ratios than adult Triomune tablets, in accordance with paediatric recommendations. Bioequivalence studies are of importance as products of poor quality should not reach the market. Formal bioequivalence of Triomune Baby and Junior was demonstrated by Cipla Ltd. at the end of 2006 (data on file). Our independent pilot bioequivalence study demonstrated similar pharmacokinetics to the branded products in 2005 already (**Chapter 3**). This has led to an earlier start of our subsequent study in HIV-infected Zambian children (**Chapter 4**), which was of benefit for those children in urgent need of Triomune Baby and Junior. The fact that we received funding from the European and Developing Countries Clinical Trials Partnership (EDCTP) for our research in the actual target population of HIV-infected African children (**Chapter 4**) underlines the high relevancy of the issue. Although the Triomune Baby and Junior antiretroviral drug ratios were shown to be appropriate for children weighing over 6 kg (**Chapter 4**) and even for those under 6 kg (unpublished data), it is of importance that the children in our trial remain to be followed for investigation of long-term safety and efficacy of the FDCs.

The correctness of WHO PAWG recommendations for drug ratios of prioritized paediatric FDCs [6] can only be confirmed by pharmacokinetic research in the actual target population of HIV-infected children. Therefore, we were invited to

present our data for Triomune Baby and Junior (**Chapter 4**) at one of the PAWG meetings in Geneva. Following successful review, WHO now recommend the antiretroviral drug ratios and the weight-band based dosing table that we used. Triomune Baby and Junior were the first paediatric FDC tablets to be approved by the United States Food and Drug Administration (FDA) and to become available for distribution under the Presidents Emergency Plan For AIDS Relief (PEPFAR) and Clinton Foundation programmes. The accessibility of Triomune Baby and Junior will drastically improve the necessary scale-up of appropriate HIV treatment for children in resource-limited countries.

It is hoped that future efforts will be made to expand the development of generic paediatric formulations with the guidance of PAWG recommendations [6]. The use of the nucleoside reverse transcriptase inhibitor (NRTI) stavudine may lead to toxicities such as lipodystrophy and peripheral neuropathy, which can be difficult to identify in children and may be irreversible. Therefore, paediatric FDCs that do not contain stavudine should be developed. Most of the generic regimens that are being developed contain the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine, while NNRTIs have a low genetic barrier for the development of resistance [7]. For those children who fail first-line NNRTI-based regimens, second-line protease inhibitor-based regimens should become available. Because it can be challenging to develop an FDC with two NRTIs and one protease inhibitor, co-packaging may be indicated.

## 2. Monitoring of HIV treatment

Provision of adequate HIV care in developing countries can be hindered by a shortage of trained personnel, complexity and costs of laboratory equipments and unreliable drug supply, especially in remote settings [8]. Staff training is required to improve the general quality of care. Patients who are not identified in time to be eligible for antiretroviral treatment remain at risk of dying from AIDS. Facilities to perform CD4 counts are needed to identify the patients eligible for treatment and to monitor the response to treatment [9]. At a large tertiary referral hospital in the northern zone of Tanzania, the Kilimanjaro Christian Medical Centre (KCMC), facilities to perform CD4 counts have recently been introduced through the National AIDS programme. Furthermore, patients on antiretroviral treatment are at risk of

side effects and laboratory abnormalities [10], but at KCMC clinical chemistry and haematology testing is only being performed for those patients who can afford to pay a small fee.

Many key anti-HIV drugs are not yet marketed in cheap generic versions. Most of the generic antiretroviral regimens that have become available in developing countries combine the NNRTI nevirapine with two NRTIs. A disadvantage of nevirapine is the low genetic barrier for the development of resistance [7]. Resistant strains against nevirapine-based regimens provided in national programmes of developing countries could be spread through the population. This is of concern, as there are few second-line options for antiretroviral therapy in resource-limited countries, where the national expenditure to health-care is restricted. Patients at risk for virological failure and resistance are those with subtherapeutic plasma concentrations of nevirapine [1, 2], which can be caused by intersubject variability in pharmacokinetics, missed doses due to inadequate drug supply, or non-adherence.

The risk of resistance development could be reduced in several ways. First of all, drug supply should be improved to prevent treatment interruptions [8]. Viral load testing would certainly help to identify those patients with an inappropriate response to treatment and thus at risk for resistance development [11], but is often unaffordable. At KCMC, viral load testing is mainly reserved for determination of the HIV status of babies that were born to HIV-positive mothers. From a pharmacological point of view, therapeutic drug monitoring (TDM) would be an important tool to identify those patients with an inappropriate exposure to drugs such as nevirapine and thus at risk for virological failure and resistance development. However, the assay technique high-performance liquid chromatography (HPLC) which is commonly used for TDM of nevirapine in the developed world [12] is too expensive for resource-poor settings. Furthermore, the required sampling technique to obtain plasma may be too complicated. To facilitate the implementation of TDM in developing countries, alternative options for sampling and assay technique are needed.

Previous studies suggest that saliva may be used as an alternative body fluid to plasma for TDM of nevirapine, implying painless and non-invasive sampling with diminished risk of HIV-transmission to health care workers, and at a lower cost [13,

14]. As alternative to HPLC, a relatively inexpensive thin layer chromatography (TLC) technique has been investigated for the detection of nevirapine in plasma [15]. We developed a cheap and simple TLC method for semi-quantitative detection of nevirapine in saliva. The assay was validated in HIV-infected Tanzanian adults on nevirapine treatment at KCMC and was found to be sensitive, specific and robust in the detection of subtherapeutic nevirapine concentrations (**Chapter 5**).

Enough capacity has been built to implement this TLC method as a tool for TDM at the HIV clinic of KCMC. In the light of cost-efficiency, it would be wise to focus on those patients suspected of inappropriate response or non-adherence to nevirapine-based treatment. Counselling of patients with a subtherapeutic nevirapine concentration should include the importance of improved adherence or an increased dose to prevent virological failure and resistance development. The effect of such interventions could be checked by repetition of TDM. Global provision of HIV care would benefit from the transfer of this TLC technique to other developing countries.

### 3. Prevention of mother-to-child transmission of HIV

Without antiretroviral treatment, the risk to transmit HIV to a baby during pregnancy or delivery is 25-48%. The introduction of highly active antiretroviral therapy (HAART) has reduced the rate of mother-to-child transmission (MTCT) to 1-2% in the developed world [16]. The mainstay of prevention of MTCT (PMTCT) programmes in low-resource settings have focussed on a single dose nevirapine to the mother shortly before delivery and to the baby after birth [17]. This simple and inexpensive intervention, reducing the risk of MTCT by 50%, is less effective than combination antiretroviral therapy [18], but was found to be cost-effective. A major disadvantage is the development of resistance in up to 70% of women [19], which may diminish the response to nevirapine in the future [20].

It is sad that combination antiretroviral therapy in pregnancy is not yet feasible in many resource-limited settings. Even the coverage of a simple PMTCT intervention such as single dose nevirapine remains low [21]. Only 30% of seropositive mother-infant pairs in a city-wide PMTCT programme in Lusaka, Zambia, received both a maternal and infant dose of nevirapine [22]. For as long as PMTCT programmes in the developing world will have to depend on the single dose

nevirapine intervention, it is useful to try and reduce the related risk for resistance development. One approach is the addition of other antiretroviral agents after delivery to inhibit viral replication. Short-courses (4-7 days) of zidovudine/lamivudine added to a single dose nevirapine significantly reduced the development of nevirapine resistance [23]. Recently, a simple adjuvant of single dose tenofovir and emtricitabine to intrapartum nevirapine was found to reduce resistance by half [24]. Because the long half-life of nevirapine is expected to contribute to the development of resistance [25], we hypothesised that a short-course of an enzyme inducer may reduce resistance development by increasing metabolism and decreasing half-life of nevirapine. Interventions of 3-7 days of phenytoin (184 mg) and even a single dose of carbamazepine (400 mg) were found to significantly reduce nevirapine half-life in healthy non-pregnant women (**Chapter 6**).

Single dose nevirapine has been recommended for use in the PMTCT programme of Tanzania from the year 2000, but was only implemented in May 2004 at the antenatal clinics in the northern zone of Tanzania. We selected the single dose carbamazepine intervention, which does not increase complexity, to be tested in Tanzanian HIV-infected pregnant women for its ability to reduce the development of nevirapine resistance (VITA-1 study). As expected, preliminary interim-results show a lower half-life in those that received an additional single dose of 400 mg carbamazepine compared to those that only received nevirapine (unpublished data). Whether this will lead to a reduction in the development of resistance has not been analysed so far. Execution of VITA-1 has been challenging with the lack of clinical trial capacity at the antenatal clinics in the Moshi area. Many women are lost to follow-up after delivery and laboratory facilities are poor. We have been missing a project car to chase those mothers not showing-up at their study visits and to transport samples from the antenatal clinics to the well-equipped biotechnology laboratory of KCMC. Another limitation is the lack of daily supervision and support of the Tanzanian PhD candidate, particularly during this author's absence. Nevertheless, as clinical trial experience and infrastructure improves, capacity is being built for future research.

One of the alternative interventions of 4-7 days zidovudine/lamivudine covers the period with subtherapeutic nevirapine concentrations below 3.0 mg/L [2] only partially, which explains that nevirapine resistance was not fully eradicated [23]. A

significant decrease in nevirapine half-life by addition of an enzyme inducer will reduce the duration of the potential zone of resistance development, but may also not be sufficient to prevent development of all nevirapine mutations. We are planning for a subsequent VITA-2 study to investigate if combining these two approaches of decreasing nevirapine half-life and co-administering other antiretroviral agents will be of added value. Our study proposal should comply with new national and WHO [26] PMTCT guidelines advising to combine the single dose of nevirapine at delivery with zidovudine from 28<sup>th</sup> week of gestation and zidovudine/lamivudine intrapartum until 1 week after delivery, if feasible.

The PMTCT field is dynamic as preferred interventions with higher activity, such as HAART, are likely to be more complex and thus less feasible for resource-limited settings. The design of future VITA studies will depend on further adjustments to PMTCT guidelines and the capacity that has been built in the mean time to implement guidelines and to perform clinical trials.

#### 4. Treatment of HIV-TB co-infection

The poverty-related communicable disease tuberculosis (TB) is the most common opportunistic infection among HIV-infected subjects worldwide, but particularly in developing countries [27]. Although co-administration of HIV and TB treatment is frequently indicated, there is a lack of knowledge about drug-drug interactions and overlap in toxicity profiles, particularly for the combination of rifampicin and protease inhibitors. When designing pharmacological trials for the optimization of HIV-TB treatment, several issues should be considered.

An international code of ethics for clinical research, the Declaration of Helsinki, has been established to assure that trials are performed in an ethical manner [28]. The declaration of Helsinki states that medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. As healthy volunteers do not benefit from a drug trial, the risks to which they are exposed can be justified only by the value of the knowledge gained by their participation [29, 30]. However, despite adequate risk estimation, it cannot be excluded that the magnitude of drug toxicity during the actual trial may be higher than expected. Outside the scope of HIV-TB research, a



Phase I trial with a new anti-CD28 monoclonal antibody (TGN1412) resulted in serious adverse events and even hospital admittance for all 6 healthy male subjects in March 2006 [31]. Within the scope of HIV-TB research, several studies in healthy volunteers were carried out to evaluate if adjusted doses of protease inhibitors would compensate for the interaction with rifampicin. Among healthy volunteers receiving once-daily rifampicin, addition of saquinavir/ritonavir [32], increased lopinavir/ritonavir doses (**Chapter 7**) and twice-daily atazanavir/ritonavir [33] resulted in an unexpectedly high incidence of adverse events. All studies were terminated prematurely. The rifampicin lead-in may have contributed to the toxicity observed. However, the studied sequence is a reflection of the clinical situation of HIV-TB co-infected patients as TB is preferably treated first.

A disadvantage of drug-drug interaction studies in HIV-infected patients is the risk for the development of subtherapeutic plasma concentrations of antiretroviral drugs. For patients who develop resistance as a result of subtherapeutic antiretroviral drug concentrations during a trial, there will be less treatment options in the future. Particularly in resource-limited settings, it will be difficult to select those patients at risk for resistance development, because of the lack of TDM facilities to monitor for subtherapeutic drug concentrations. The fact that there is no network to perform clinical drug trials in HIV-TB co-infected patients in Europe has limited the outcome of our study evaluating the clinical experience with the combined use of lopinavir/ritonavir and rifampicin (**Chapter 8**). As only a minority of physicians appeared to follow recommendations and treatment was not standardised across the institutions involved, it was difficult to draw conclusions. We would have learned more from **Chapter 8** if expertise had been gathered.

Taking into account these experiences, it would be best to investigate the combination of rifampicin and antiretroviral drugs in HIV-TB co-infected patients in the developing world. A requirement would be that patients with an inappropriate exposure to antiretroviral drugs and thus at risk for resistance development will be identified in time. In case there is not enough funding or capacity to implement current methods for TDM in developing countries, efforts should be made to develop alternative methods that are feasible. A simple and inexpensive TLC method has already been developed for the determination of nevirapine (**Chapter 5**), but not yet for the determination of protease inhibitors. In 2008, a study investigating the

combination of rifampicin-containing tuberculostatic treatment and first-line antiretroviral treatment (tenofovir, emtricitabine and efavirenz) will be started at a large referral hospital for HIV-TB co-infected patients, Kibon'goto National Tuberculosis Hospital (KNTH), in the northern zone of Tanzania. It is hoped that hereby capacity is being built for future studies investigating the combination of rifampicin and second-line antiretroviral treatment, which is often based on protease inhibitors. As a follow-up of **Chapters 7 and 8** it would be interesting to study a rifampicin lead-in followed by addition of standard lopinavir/ritonavir doses in HIV-TB co-infected patients. This should happen under online TDM of lopinavir/ritonavir concentrations to be able to increase the dose if necessary.

## 5. North-South collaboration in clinical pharmacological research

Clinical pharmacological research questions that are specifically relevant to developing countries are not always addressed in countries with a high-standard of health care. A North-South collaboration between partners from the developed and developing world can facilitate pharmacological research in resource-poor countries. Research institutes from the developed world can contribute their experience and institutes from the developing world are needed for the provision of ethics approval, study personnel and patients. The collaboration between two European and two African institutes in this thesis was achieved by networking. One of the preceding pharmacology PhD students at the Radboud University Nijmegen Medical Centre (RUNMC) in the Netherlands, Alina Bergshoeff, was involved in the paediatric European network for treatment of AIDS (PENTA). Within this PENTA network, RUNMC has collaborated with the Medical Research Council (MRC), Clinical Trials Unit (CTU) in London, United Kingdom. MRC CTU was also collaborating with the University Teaching Hospital (UTH) in Lusaka, Zambia and asked the RUNMC to join the network. Nijmegen University has a history of 37 years of collaboration with the Tanzanian Health system. A decade ago, RUNMC was very closely involved in the development of the Medical College at KCMC in Moshi, Tanzania. This has been the basis for a research collaboration between the two institutes.

When looking at the output of this thesis, it can be said that the collaboration between European and African institutes has been successful. The large pharmacokinetic trial in Zambian HIV-infected children (**Chapter 4**) has benefitted from the pharmacological

experience of our department at RUNMC and from the clinical trial experience of MRC CTU. When this so-called CHAPAS1 study was started in Zambia, the infrastructure to perform clinical trials was already in place. The same study clinic and nurses were involved in the previous so-called CHAP trial (**Chapter 2**, [34]). In contrast, execution of the VITA-1 study, as a follow-up to **Chapter 6**, trying to optimize PMTCT in Tanzania, appears to be much more complicated. There was a general lack of clinical trial capacity when VITA-1 was started. In addition, MRC CTU with a lot of clinical trial experience in resource-limited settings has not been involved so far.

Pharmaceutical industries that are traditional sponsors of drug trials in the developed world are often not interested to sponsor research for developing countries. Sponsorships should come from international governmental organisations such as the European Union, The Bill Gates Foundation, G8, WHO and the Joint United Nations Programme on HIV/AIDS (UNAIDS). Most of the research in this thesis was funded by EDCTP and by the Poverty Related Infection Oriented Research (PRIOR) network that was established by a grant from the Netherlands Foundation for the Advancement of Tropical Research (NWO-WOTRO) and the Netherlands Foundation for Health Research and Development (ZonMw).

## Conclusions and future perspectives

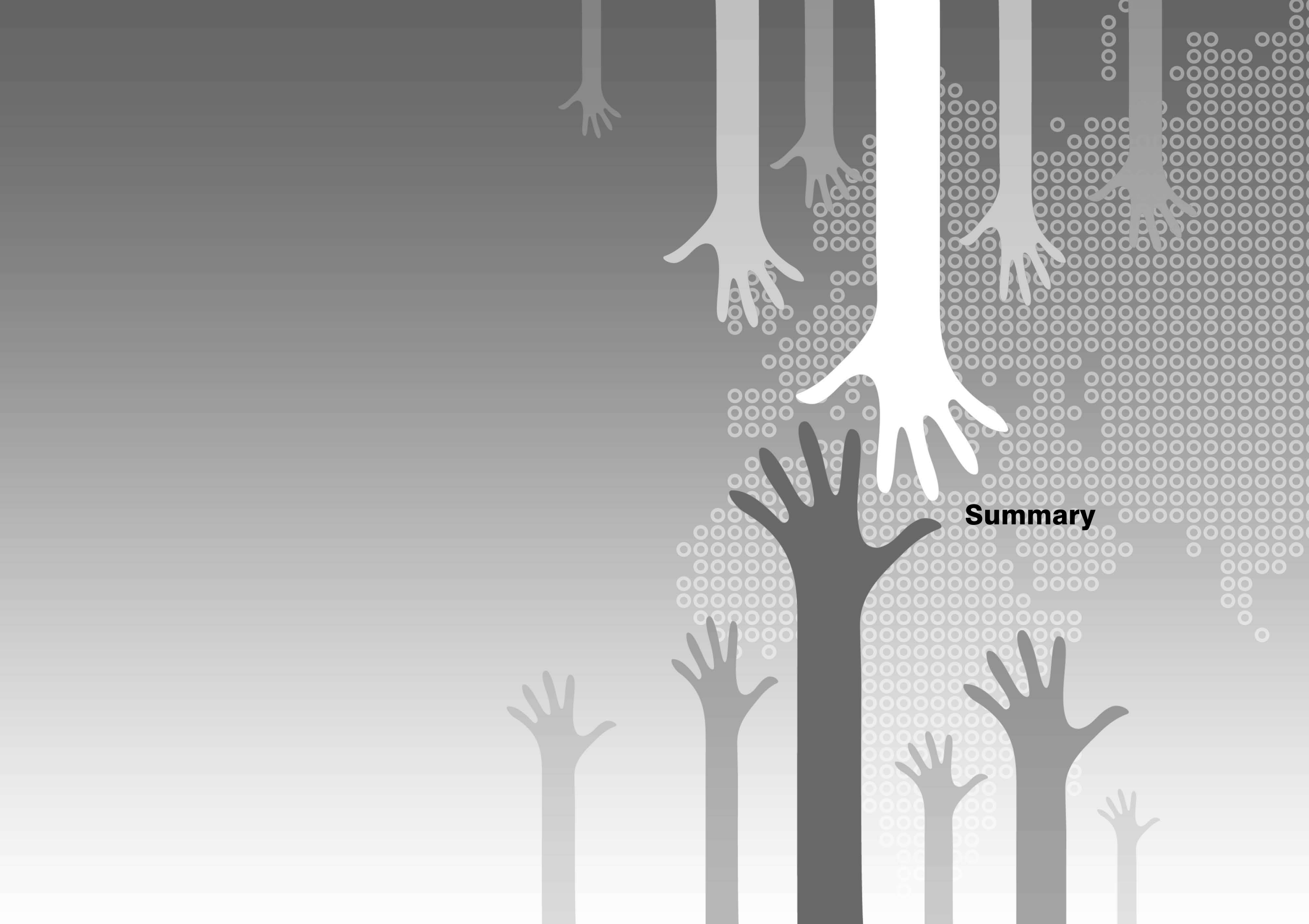
In conclusion, our North-South collaboration between research partners from the developed and developing world has improved the knowledge about clinical pharmacology of HIV treatment that is specifically relevant for resource-limited countries. Although the first affordable and appropriate paediatric FDC tablets recently became available for HIV-infected children in developing countries, future efforts should be made to expand the development of generic paediatric formulations. It is hoped that the cheap and simple TLC method that was developed for the detection of nevirapine in saliva is implemented as a tool for TDM in resource-limited countries. For as long as PMTCT programmes in the developing world will have to depend on the single dose nevirapine intervention, the search for interventions to reduce the risk of resistance development should be continued. Moreover, future research is needed in HIV-TB co-infected patients in the developing world to investigate the co-administration of HIV and TB treatment. The key to

success of a North-South collaboration in clinical pharmacological research of HIV treatment is the complementary contribution of different partners. Adequate funding is required to build the capacity to perform clinical trials in the developing world and experienced scientists are needed to train and supervise the local study staff.

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**Summary**

## Introduction

This thesis presents the first output of a North-South (Europe-Africa) collaboration in clinical pharmacological research of HIV treatment. **Part I** deals with the optimization of paediatric regimens for the scale-up of HIV treatment in resource-limited countries. **Part II** focuses on therapeutic drug monitoring (TDM) in HIV-infected adults and the optimization of a cost-effective strategy for the reduction of mother-to-child transmission (MTCT) of HIV in low-income countries. **Part III** describes the challenges of combined treatment with lopinavir/ritonavir and rifampicin for HIV-tuberculosis (TB) co-infection.

### I. Clinical pharmacology in HIV-infected children

The brief review in **Chapter 1** focuses on general characteristics relating to pharmacokinetic processes (absorption, distribution, metabolism, excretion) in the paediatric population, followed by recent examples of antiretroviral drug pharmacokinetics in HIV-infected children. Pharmacokinetics of antiretroviral drugs are highly variable in the paediatric population as children mature and grow rapidly and individually until they are adults. It was unexpectedly found that exposure to lopinavir is decreased in the first 6 months of life. In addition, recommended dosing of lamivudine leads to lower exposure in children younger than 6 years of age. Recent findings of subtherapeutic efavirenz concentrations in children suggest that paediatric dose recommendations should be re-evaluated. There is a general shortage of pharmacokinetic data in HIV-infected children. Selected pharmacology studies should be undertaken to improve paediatric dose guidance of existing antiretroviral drugs. TDM is a useful tool to optimize treatment in HIV-infected children. However, more data are needed to establish child-specific reference values.

**Chapter 2** describes the exposure to nevirapine in HIV-infected African children treated with divided adult fixed dose combination (FDC) tablets (Triomune) in routine outpatient settings. Of the 127 children, 23 (18%) had a subtherapeutic random nevirapine plasma concentration ( $<3.0$  mg/L). Only 1 (3%) of those prescribed  $\geq 300$  mg/m<sup>2</sup>/day nevirapine had subtherapeutic concentrations compared with 22 (23%) of those prescribed  $<300$  mg/m<sup>2</sup>/day. Lower nevirapine concentrations were independently associated with lower prescribed dose/m<sup>2</sup>, lower height-for-age, and higher body mass index-for-age. Most children with

subtherapeutic nevirapine concentrations were taking half or quarter FDC tablets. It was concluded that currently available adult FDC tablets are not well suited for children, particularly at younger ages. Development of appropriate paediatric FDC tablets is essential if antiretroviral therapy is to be made widely available to children in resource-limited settings.

Two generic paediatric FDC tablets (Triomune Baby & Junior) were developed with higher nevirapine to stavudine and lamivudine ratios than the adult FDC tablet (Triomune) in accordance with paediatric dose recommendations. In **Chapter 3**, the pharmacokinetics of Triomune Baby & Junior were compared to the branded products. This pilot study in six healthy males shows that the pharmacokinetic profiles of stavudine, lamivudine and nevirapine in the generic paediatric FDC tablets are similar to the branded products. Based on these results, it is acceptable to test the pharmacokinetics and dosing requirements of Triomune Baby & Junior in HIV-infected children.

**Chapter 4** describes the pharmacokinetics of the two generic paediatric FDC tablets Triomune Baby & Junior in a target population of 71 African HIV-infected children. After treatment of the children according to weight-bands for at least 4 weeks, a 12-hour pharmacokinetic curve was recorded. Nevirapine plasma concentrations were higher but more variable than those in adults. The nevirapine trough concentration was subtherapeutic ( $<3.0$  mg/L) in four (6%) children. Pharmacokinetic parameters of stavudine and lamivudine were comparable to those in adults. There was no evidence of a difference in nevirapine exposure across weight-bands, while the difference in stavudine and lamivudine exposure was driven by the single weight-band with an unequal morning and evening dose (15- $<20$ kg). As nevirapine underdosing is of greater concern than overdosing, it was concluded that the Triomune Baby & Junior ratio is appropriate for children weighing 6kg and over. Further research is required for children under 6kg.

### II. Clinical pharmacology in HIV-infected adults

In **Chapter 5**, the option to perform TDM of nevirapine in resource-limited settings was explored. A simple and inexpensive thin layer chromatography (TLC) assay was developed for semi-quantitative detection of nevirapine saliva concentrations. The method was validated in a target population of 300 African HIV-infected adults.

Paired plasma and saliva nevirapine concentrations were assayed by high-performance liquid chromatography (HPLC). Saliva concentrations were also assayed by TLC. Nine percent of the subjects had a subtherapeutic plasma nevirapine concentration. The median saliva/plasma nevirapine concentration ratio was 0.51. The TLC assay was found to be sensitive, specific and robust in detection of subtherapeutic nevirapine concentrations in saliva of African HIV-infected adults. It is an attractive alternative to HPLC for TDM of nevirapine in resource-limited settings.

Single-dose nevirapine to prevent MTCT of HIV is associated with the development of nevirapine resistance, probably due to its long elimination half-life in combination with a low genetic barrier to resistance. The pilot study in **Chapter 6** aimed to identify enzyme inducers to reduce the half-life of nevirapine. After administration of a single 200-mg dose of nevirapine to 36 healthy non-pregnant women in both period 1 and 2, blood was sampled twice-a-week for 21 days to determine concentrations of nevirapine. In period 2 additional interventions (single dose carbamazepine, phenobarbital or phenytoin; phenytoin for 3 or 7 days; St John's Wort, vitamin A or cholecalciferol for 14 days) were administered to all subjects except for the control group. In three intervention groups T-half ratio (nevirapine half-life in period 2 / half-life in period 1) differed significantly from the control group: single 400-mg dose of carbamazepine, 184mg phenytoin once daily for 3 or 7 days. The mean decrease in nevirapine half-life in period 2 was 35.3, 38.2 and 35.9%, in these groups respectively. It was concluded that these interventions should be tested in the setting of single-dose nevirapine for the prevention of MTCT (PMTCT) of HIV to reduce the development of nevirapine resistance.

### III. Clinical pharmacology in HIV-TB co-infection

Previous research in healthy volunteers has demonstrated that increased doses of lopinavir/ritonavir soft gel capsules compensate for the interaction with rifampicin. The pharmacokinetic interaction study in **Chapter 7** shows a high incidence of adverse events in 11 healthy volunteers treated with a higher than standard dose of the new lopinavir/ritonavir tablets in combination with rifampicin. No major complaints occurred when 600 mg rifampicin was given only. However, the study had to be terminated prematurely after addition of 600/150 or 800/200 mg lopinavir/ritonavir twice daily. Eight subjects suffered from both nausea and vomiting, one

from nausea only, and one from vomiting only. Moreover, increases in AST/ALT levels were reported in all subjects. All levels returned to normal within six weeks. The observed toxicity may be explained by three facts. Firstly, rifampicin was introduced prior to lopinavir/ritonavir; secondly, the adjusted dose of lopinavir/ritonavir tablets was not escalated; and thirdly, the new lopinavir/ritonavir tablet formulation was used instead of the capsule formulation. It was advised that further studies investigating the interaction between rifampicin and lopinavir/ritonavir tablets be performed in the actual target population of HIV-TB co-infected patients rather than in healthy volunteers.

**Chapter 8** describes the clinical experience with the combined use of lopinavir/ritonavir and rifampicin in the Netherlands. Thirty-four adult HIV-infected patients on combined treatment were selected from the Dutch ATHENA cohort for retrospective analysis. Overall, 15% used a recommended increased dose of lopinavir/ritonavir, 82% were on the soft gel capsule formulation of lopinavir/ritonavir and 68% had a lead-in with rifampicin. Of the 23 patients on a non-adjusted dose of lopinavir/ritonavir, 4 out of 6 (67%) had a subtherapeutic lopinavir trough plasma concentration (<1.0 mg/L) and 3 out of 8 (38%) had a detectable viral load ( $\geq 50$  copies/ml) after at least 4 months of concomitant treatment. Two out of 5 (40%) patients on a recommended increased dose of lopinavir/ritonavir prematurely stopped the drug combination because of adverse events vs. 4 out of 23 (17%) on a non-adjusted dose and 1 out of 6 (17%) on a slightly increased dose. An adequately powered prospective study in HIV-TB co-infected patients is needed to investigate the consequences of inferior lopinavir exposure due to non-adjusted doses of lopinavir/ritonavir and to determine the tolerability of recommended increased doses.

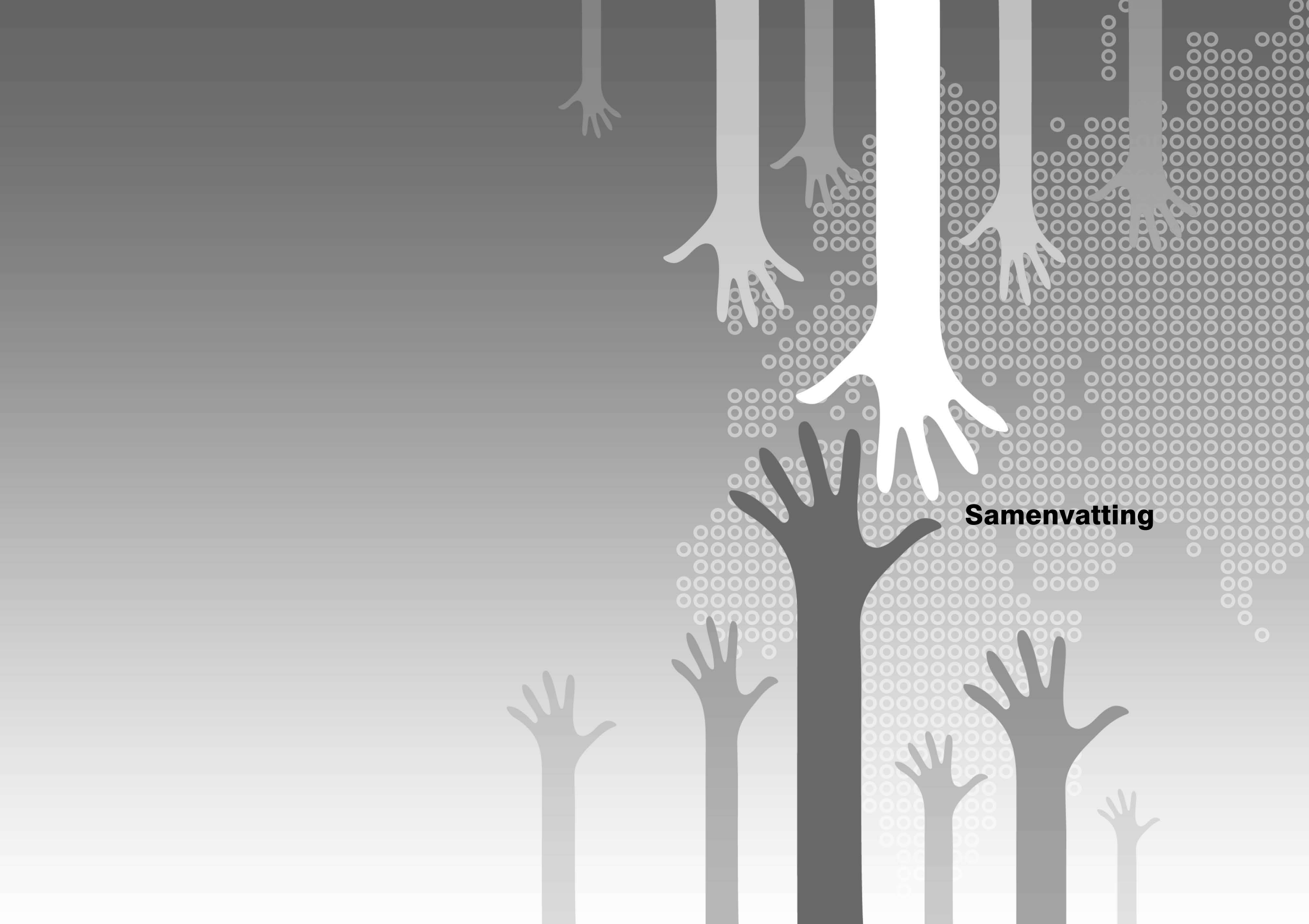
### General discussion

In the general discussion the following issues that are relevant for developing countries are discussed: access to HIV treatment, monitoring of HIV treatment, PMTCT of HIV, treatment of HIV-TB co-infection, and North-South collaboration in clinical pharmacological research.

Although the first affordable and appropriate paediatric FDC tablets recently became available for HIV-infected children in developing countries, future efforts



should be made to expand the development of generic paediatric formulations. It is hoped that the cheap and simple TLC method that was developed for the detection of nevirapine in saliva will soon be implemented in resource-limited countries. For as long as PMTCT programmes in the developing world will have to depend on the single dose nevirapine intervention, the search for interventions to reduce the risk of resistance development should be continued. Moreover, future research is needed in HIV-TB co-infected patients in the developing world to investigate the co-administration of HIV and TB treatment. The key to success of a North-South collaboration in clinical pharmacological research of HIV treatment is the complementary contribution of different partners.



**Samenvatting**

## Introductie

Dit proefschrift laat de eerste resultaten zien van een Noord-Zuid samenwerking tussen Europa en Afrika met betrekking tot klinisch farmacologisch onderzoek van HIV behandeling. **Deel I** gaat over de optimalisering van preparaten om kinderen met HIV in ontwikkelingslanden te kunnen behandelen. De focus van **Deel II** ligt bij het monitoren van geneesmiddelconcentraties (therapeutic drug monitoring, TDM) in volwassenen met HIV. Daarnaast is er aandacht voor de optimalisering van een kosteneffectieve strategie voor de vermindering van HIV overdracht van moeder op kind (mother-to-child transmission, MTCT) in ontwikkelingslanden. **Deel III** beschrijft de uitdagingen van gecombineerde behandeling met lopinavir/ritonavir en rifampicine in geval van HIV-tuberculose (TB) dubbelinfectie.

## I. Klinische farmacologie in HIV geïnfecteerde kinderen

**Hoofdstuk 1** geeft een beknopt overzicht van de algemene kenmerken van farmacokinetische processen (opname, verdeling, afbraak, uitscheiding) in kinderen, gevolgd door recente voorbeelden van de farmacokinetiek van antiretrovirale geneesmiddelen in HIV geïnfecteerde kinderen. De farmacokinetiek van antiretrovirale geneesmiddelen in kinderen is sterk variabel aangezien kinderen zich individueel ontwikkelen en groeien totdat ze volwassen zijn. Er is onverwacht aangetoond dat de blootstelling aan lopinavir is verminderd in kinderen jonger dan 6 maanden. Daarnaast leidt de aanbevolen dosering van lamivudine tot een lagere blootstelling in kinderen jonger dan 6 jaar. Recente bevindingen van te lage, oftewel subtherapeutische, efavirenz concentraties in kinderen laten zien dat de aanbevolen kinderdosering van efavirenz opnieuw geëvalueerd zou moeten worden. Er is een algemeen tekort aan farmacokinetische data in kinderen met HIV. Zorgvuldig gekozen farmacologische onderzoeken zouden uitgevoerd moeten worden om de aanbeveling van kinderdoseringen van bestaande antiretrovirale geneesmiddelen te verbeteren. Het monitoren van geneesmiddelconcentraties zou de behandeling van kinderen met HIV kunnen verbeteren. Desondanks zijn er meer gegevens nodig om specifieke referentiewaarden voor kinderen te bepalen.

**Hoofdstuk 2** beschrijft de blootstelling aan nevirapine in HIV geïnfecteerde Afrikaanse kinderen die in de thuissituatie behandeld worden met gedeelde vaste dosis combinatietabletten (fixed dose combination, FDC) voor volwassenen

(Triomune). Van de 127 kinderen hadden er 23 (18%) een subtherapeutische willekeurige nevirapine bloedconcentratie ( $<3.0$  mg/L). Slechts 1 (3%) van de kinderen die  $\geq 300$  mg/m<sup>2</sup> nevirapine per dag kregen had een subtherapeutische concentratie in vergelijking met 22 (23%) van de kinderen die  $<300$  mg/m<sup>2</sup> nevirapine per dag gebruikten. Lagere nevirapine concentraties waren onafhankelijk geassocieerd met een lagere voorgeschreven dosering per m<sup>2</sup>, een lagere lengte voor de leeftijd en een hogere body mass index voor de leeftijd. De meeste kinderen met subtherapeutische nevirapine concentraties werden behandeld met halve of kwart FDC tabletten. Er werd geconcludeerd dat de huidige beschikbare FDC tabletten voor volwassenen niet geschikt zijn voor (met name jonge) kinderen. De ontwikkeling van FDC kindertabletten is essentieel voor het algemeen beschikbaar maken van antiretrovirale therapie voor kinderen in ontwikkelingslanden.

Er zijn twee generieke FDC kindertabletten (Triomune Baby & Junior) ontwikkeld die voldoen aan de aanbevolen kinderdoseringen. Deze kindertabletten bevatten relatief meer nevirapine en relatief minder stavudine en lamivudine in vergelijking tot de FDC tablet voor volwassenen (Triomune). In **Hoofdstuk 3** werd de farmacokinetiek van Triomune Baby & Junior vergeleken met de merkpreparaten. Dit proefonderzoek in zes gezonde mannen laat zien dat de farmacokinetische profielen van stavudine, lamivudine en nevirapine in de generieke kindertabletten vergelijkbaar zijn met de merkpreparaten. Gebaseerd op deze resultaten kan gesteld worden dat het acceptabel is om de farmacokinetiek en de vereiste dosering van Triomune Baby & Junior in HIV geïnfecteerde kinderen te onderzoeken.

**Hoofdstuk 4** beschrijft de farmacokinetiek van de twee generieke FDC kindertabletten Triomune Baby & Junior in een doelpopulatie van 71 Afrikaanse HIV geïnfecteerde kinderen. Na behandeling van de kinderen aan de hand van gewichtsgroepen voor tenminste 4 weken werden er diverse bloedmonsters afgenomen gedurende 12 uur. Nevirapine concentraties waren hoger en meer wisselend dan die in volwassenen. De nevirapine dalconcentratie was subtherapeutisch ( $<3.0$  mg/L) in vier (6%) kinderen. De farmacokinetische parameters van stavudine en lamivudine waren vergelijkbaar met die in volwassenen. Er was geen bewijs voor een verschil in nevirapine blootstelling tussen de gewichtsgroepen, terwijl het verschil in blootstelling aan stavudine en lamivudine werd veroorzaakt door de enige gewichtsgroep met ongelijke ochtend- en avonddosering (15- $<20$ kg).

Omdat nevirapine onderdosering van grotere zorg is dan overdosering, werd geconcludeerd dat de geneesmiddelenratio in Triomune Baby & Junior geschikt is voor kinderen van tenminste 6kg. Meer onderzoek is nodig voor kinderen met een gewicht van minder dan 6kg.

## II. Klinische farmacologie in HIV geïnficeerde volwassenen

In **Hoofdstuk 5** werd bekeken of er een mogelijkheid bestaat om concentraties van nevirapine te monitoren (therapeutic drug monitoring, TDM) in een omgeving met beperkte middelen. Een eenvoudige en goedkope dunne laag chromatografie (DLC) methode werd ontwikkeld voor semikwantitatieve detectie van nevirapine speekselconcentraties. De methode werd gevalideerd in een doelpopulatie van 300 Afrikaanse volwassenen met HIV. Gepaarde bloed- en speekselconcentraties van nevirapine werden gemeten met HPLC (high-performance liquid chromatography). Speekselconcentraties werden tevens bepaald met DLC. Negen procent van de patiënten had een subtherapeutische bloedconcentratie van nevirapine. De mediane speeksel/bloed concentratieratio van nevirapine was 0.51. De ontwikkelde DLC is een gevoelige, specifieke en robuuste methode voor de detectie van subtherapeutische nevirapine concentraties in speeksel van Afrikaanse HIV geïnficeerde volwassenen. Het is een aantrekkelijk alternatief voor HPLC om TDM van nevirapine in ontwikkelingslanden mogelijk te maken.

Een éénmalige dosis nevirapine, zoals gebruikt in Afrika ter voorkoming van moeder-op-kind transmissie van HIV, is geassocieerd met de ontwikkeling van resistentie tegen nevirapine. Dit is waarschijnlijk het gevolg van de lange eliminatiehalfwaardetijd van nevirapine in combinatie met een lage barrière tegen resistentie. Het proefonderzoek in **Hoofdstuk 6** was gericht op de zoektocht naar enzyminductoren om de eliminatiehalfwaardetijd van nevirapine te bekorten. Na toediening van een éénmalige 200-mg dosis van nevirapine aan 36 gezonde, niet-zwangere vrouwen in zowel periode 1 als 2, werd twee maal per week gedurende 21 dagen bloed afgenomen ter bepaling van nevirapine concentraties. In periode 2 werden additionele interventies (éénmalige dosis van carbamazepine, fenobarbital of fenytoïne; fenytoïne gedurende 3 of 7 dagen; St Janskruid thee, vitamine A of vitamine D gedurende 14 dagen) toegediend aan alle personen behalve de controlegroep. In drie interventiegroepen verschilde T-half ratio

(nevirapine halfwaardetijd in periode 2 / halfwaardetijd in periode 1) significant van de controlegroep: éénmalige 400-mg dosis van carbamazepine, 184mg fenytoïne één maal daags gedurende 3 dagen of 7 dagen. De gemiddelde daling in nevirapine halfwaardetijd in periode 2 was respectievelijk 35.3, 38.2 and 35.9% in deze groepen. Deze interventies zouden in Afrika getest moeten worden in de setting van een éénmalige dosis nevirapine ter preventie van moeder-op-kind transmissie met het doel de ontwikkeling van nevirapine resistentie te verminderen.

## III. Klinische farmacologie bij HIV-TB dubbelinfectie

Voorafgaand onderzoek in gezonde vrijwilligers heeft aangetoond dat verhoogde doseringen van lopinavir/ritonavir zachte gel capsules compenseren voor de interactie met rifampicine. Het farmacokinetische interactieonderzoek in **Hoofdstuk 7** laat een hoge incidentie van bijwerkingen zien in 11 gezonde vrijwilligers bij gecombineerd gebruik van rifampicine en een hogere dan standaard dosering van de nieuwe lopinavir/ritonavir tabletten. Rifampicine in een dosering van 600 mg werd goed verdragen. Maar het onderzoek moest vroegtijdig worden stopgezet na toevoeging van 600/150 of 800/200 mg lopinavir/ritonavir twee maal daags. Acht vrijwilligers waren misselijk en moesten braken, één vrijwilliger was alleen misselijk en één moest alleen braken. Daarnaast werden verhoogde ASAT/ALAT waarden gerapporteerd in alle vrijwilligers. Alle waarden keerden binnen 6 weken terug naar normaal. Er zijn drie mogelijke oorzaken te noemen voor de geobserveerde toxiciteit. Allereerst werd rifampicine eerder geïntroduceerd dan lopinavir/ritonavir. Ten tweede werd de aangepaste dosering van de lopinavir/ritonavir tabletten niet geleidelijk verhoogd. Ten derde werd de nieuwe tabletformulering van lopinavir/ritonavir gebruikt in plaats van de capsuleformulering. Het advies was om vervolgonderzoek naar de interactie tussen rifampicine en lopinavir/ritonavir tabletten uit te voeren in de werkelijke doelpopulatie van HIV-TB geïnficeerde patiënten in de plaats van in gezonde vrijwilligers.

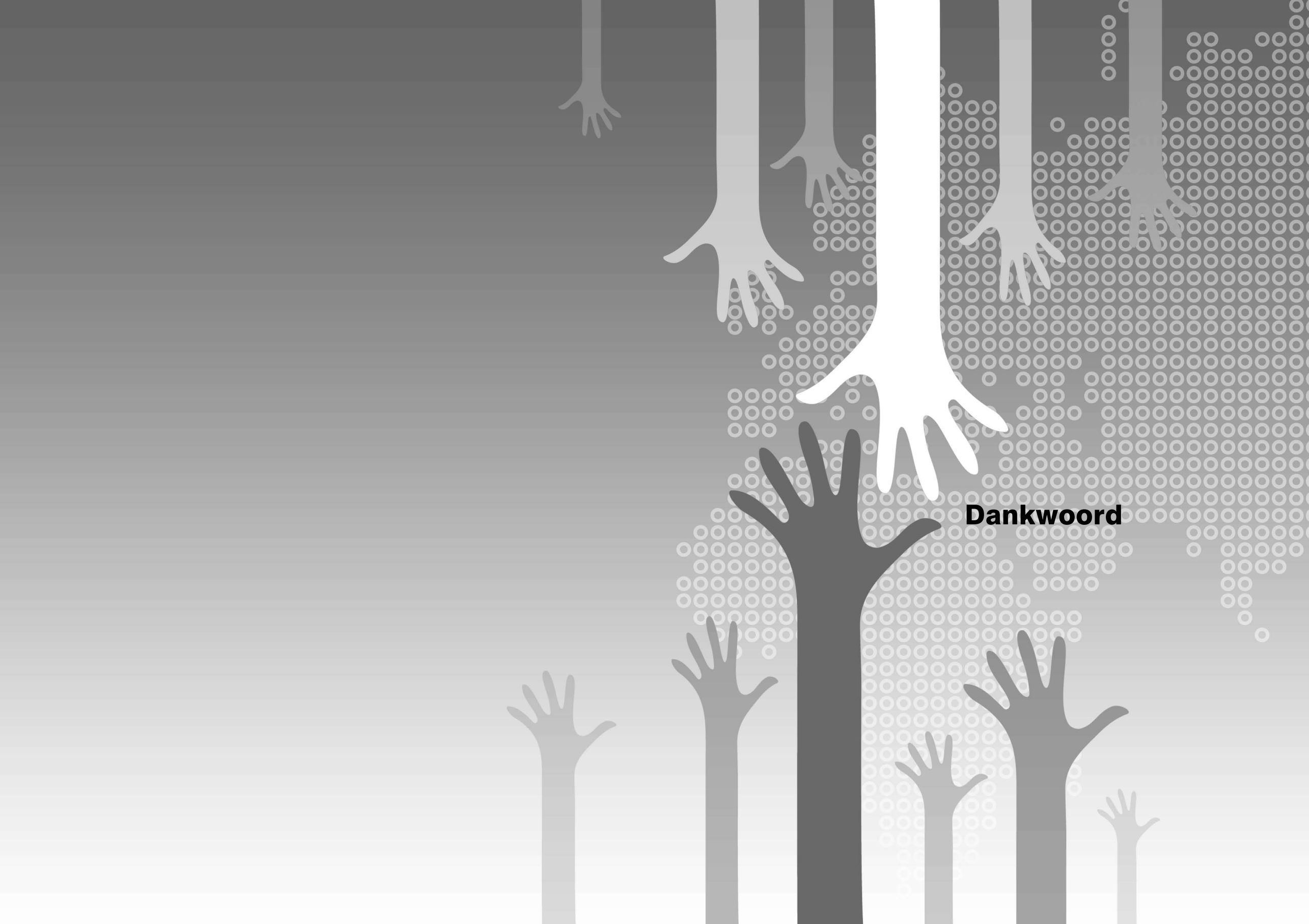
**Hoofdstuk 8** beschrijft de klinische ervaring met het gecombineerd gebruik van lopinavir/ritonavir en rifampicine in Nederland. Vierendertig volwassen HIV geïnficeerde patiënten behandeld met bovenstaande combinatie werden voor retrospectieve analyse geselecteerd uit het Nederlandse ATHENA cohort. Van de 34 patiënten, gebruikte 15% een aanbevolen verhoogde dosering van lopinavir/ritonavir en 82% de capsuleformulering van lopinavir/ritonavir. In 68% van de

patiënten werd rifampicine eerder geïntroduceerd dan lopinavir/ritonavir. Van de 23 patiënten op een niet-aangepaste dosering van lopinavir/ritonavir, hadden 4 van de 6 (67%) een subtherapeutische lopinavir dalconcentratie ( $<1.0$  mg/L) en 3 van de 8 (38%) een detecteerbare virale load ( $\geq 50$  kopieën/ml) na gecombineerde behandeling voor tenminste 4 maanden. Twee van de 5 (40%) patiënten op een aanbevolen verhoogde dosering van lopinavir/ritonavir stopten vroegtijdig met de geneesmiddelcombinatie in verband met bijwerkingen vs. 4 van de 23 (17%) op een niet-aangepaste dosering en 1 van de 6 (17%) op een licht verhoogde dosering. Een groot prospectief onderzoek in HIV-TB geïnfecteerde patiënten is nodig naar de consequenties van te lage lopinavir blootstelling door niet-aangepaste doseringen van lopinavir/ritonavir en naar de verdraagbaarheid van aanbevolen verhoogde doseringen.

## Algemene discussie

Het proefschrift wordt afgesloten met een discussie over punten die relevant zijn voor ontwikkelingslanden: toegang tot HIV behandeling, monitoren van HIV behandeling, preventie van moeder-op-kind transmissie van HIV (PMTCT), behandeling van HIV-TB dubbelinfectie, en Noord-Zuid samenwerking met betrekking tot klinisch farmacologisch onderzoek.

Ondanks het feit dat sinds kort de eerste goedkope en adequate FDC kindertabletten beschikbaar zijn voor HIV geïnfecteerde kinderen in ontwikkelingslanden, is het van groot belang dat de ontwikkeling van generieke kinderformuleringen in de toekomst wordt voortgezet. Het is te hopen dat de eenvoudige en goedkope DLC methode die werd ontwikkeld voor de detectie van nevirapine in speeksel binnenkort zal worden geïmplementeerd in ontwikkelingslanden. Voor zolang PMTCT programma's in ontwikkelingslanden afhankelijk zijn van een éénmalige dosis nevirapine, zou de zoektocht naar interventies voor het verkleinen van het risico op resistentieontwikkeling moeten voortduren. Daarnaast zou er een plek moeten zijn voor verder onderzoek in HIV-TB geïnfecteerde patiënten in ontwikkelingslanden naar de gecombineerde behandeling van HIV en TB. De sleutel tot succes van een Noord-Zuid samenwerking met betrekking tot klinisch farmacologisch onderzoek van HIV behandeling is de complementaire bijdrage van verschillende onderzoekspartners.



**Dankwoord**

Al weer vier jaar geleden begon ik met mijn promotieonderzoek bij de afdeling Apotheek/Klinische Farmacie van het UMC St Radboud. Hoewel ik het grootste deel van de tijd in Nijmegen was, ben ik regelmatig voor 4-8 weken in Tanzania en Zambia geweest. Daarnaast bestond er een intensieve samenwerking met een onderzoeksgroep in Engeland. Het reizen en samenwerken met andere culturen binnen mijn promotietraject heb ik ervaren als een zeer geslaagd experiment! Het is me een genoegen om een woord van dank te richten tot allen die een bijdrage hebben geleverd aan het onderzoek in dit proefschrift.

Prof. dr. Hekster, promotor, beste Chiel, ik ben onder de indruk van je kennis, creativiteit en toegankelijkheid! Je was altijd op de achtergrond aanwezig om de onderzoeken in goede banen te leiden, wat ik als zeer prettig heb ervaren.

Dr. Burger, co-promotor, beste David, ik had me geen betere begeleider kunnen wensen! Doordat je deur ten alle tijden voor me openstond, werden mijn vragenlijstjes steeds binnen no-time weggewerkt. Dit heeft mij geholpen om efficiënt te kunnen werken. Ondanks je overvolle agenda, ben je een man op wie je kunt bouwen en dat siert je. Je passie voor NEC heb ik helaas niet met je kunnen delen, maar wel je hart voor Afrika. Fantastisch dat je onderzoekslijn een bijdrage levert aan de HIV behandeling waar het zó hard nodig is!

De analisten van het Laboratorium Klinische Farmacie wil ik hartelijk bedanken voor het uitvoeren van de vele metingen en voor de interesse in het onderzoek achter de getallen. Zonder jullie was ik nooit zo ver gekomen in de afgelopen vier jaar. Peter Diebels, (destijds) hoofdanalist, ik vond het erg plezierig om met je samen te werken. Knap hoe je, ondanks de drukte op het Laboratorium, het meten van de onderzoeksmonsters telkens weer snel ingepland kreeg. Rob Aarnoutse, hoofd van het Laboratorium, als begeleider van Hanneke heb ik zeker ook veel van jouw kennis mogen opsteken. Bedankt hiervoor!

Oud-collega HIV onderzoekers: Jackie, ik wil je danken voor alles wat ik van je heb geleerd! Geweldig dat je een eenvoudige en goedkope methode hebt ontwikkeld voor de bepaling van nevirapine in speeksel. Samen met Liselotte was je van onschatbare waarde bij het tot stand komen van hoofdstuk 5. Manon, je ging me nog geen jaar geleden voor in het promotietraject, bedankt voor de tips! Ik wens je

veel succes met je opleiding tot klinisch farmacoloog en je verdere onderzoeks-carrière.

Collega-promovendi: Hanneke, ik vond het erg gezellig om een kamer met je te delen. Bedankt voor je enorme bijdrage aan hoofdstuk 7 en 8 naar de gecombineerde behandeling van HIV en tuberculose. Ik vind het knap dat je als eerste tuberculose onderzoeker binnen de afdeling zo'n mooie studies hebt uitgevoerd in Nederland en Indonesië. Veel succes met de verdediging van je proefschrift en met je nieuwe baan. En dan de mannen, Roger & Matthijs, dank voor jullie gezelligheid en succes met het afronden van jullie onderzoek.

Anita Huisman en Angela Colbers, hartelijk dank voor jullie gezelschap en jullie hulp bij het oplossen van vele praktische problemen met betrekking tot de onderzoeken in Nederland en Afrika.

Graag wil ik alle collega's uit de apotheek bedanken voor de interesse in mijn onderzoek en de gezelligheid tijdens de pauzes.

I would like to thank Prof. Chifumbe Chintu for giving me the opportunity to work at UTH in Lusaka, Zambia.

Prof. Diana Gibb, it has been a pleasure to work with you and learn about paediatrics. I am impressed by your agenda and the way you keep many relevant projects running in resource-poor countries.

I want to thank Veronica Mulenga for making me feel at home at UTH.

Desiré Kabamba, thank you for your help with the CHAPAS1 PK study (Chapter 4). I very much enjoyed the joint presentation of our study results in Los Angeles.

Fiona Ewings & Sarah Walker, thanks so much for your help with statistics and writing.

Margaret Thomason & Fiona Ewings, I very much enjoyed our trip to the Victoria falls! I still have to laugh when I think about all the funny experiences we had.



Further thanks to all the colleagues from the CHAPAS team at UTH (Lusaka, Zambia) and MRC CTU (London, UK). It has been absolutely great to work with you in the CHAPAS clinic.

André van der Ven dank ik voor de mogelijkheid die me geboden werd om promotieonderzoek te doen binnen PRIOR.

I would like to thank Prof. Shao for giving me the opportunity to work at KCMC in Moshi, Tanzania.

Special thanks to my counterpart Eva Muro for the pleasant cooperation and for the hard work collecting our study samples. I hope you enjoyed your stay in the Netherlands just as much as I enjoyed working in Tanzania. Good luck with the (VITA) studies and the rest of your PhD!

Werner Schimana, thank you for your time to be our local supervisor at KCMC.

I thank dr. Frank Mosha for his warm hospitality and local support of our research projects at KCMC.

Liselotte Wolters, je doet je bijnaam "the flower of PRIOR" eer aan! Zonder jouw hulp op het Laboratorium in Tanzania waren onze projecten niet mogelijk geweest. Ook wil ik je bedanken voor de gezelligheid tijdens mijn verblijf in Tanzania.

Mirjam Oudijk & Minke Huibers, hartelijk dank voor jullie hulp als stagiaires bij de VITA1 studie. Leuk om te horen dat Afrika jullie blijvende interesse heeft.

Clara Blaauw, Mieke Daalderop en Carla Ysenbrandt dank ik voor de goede organisatie van mijn reizen naar Afrika.

And to all the PRIOR colleagues: Asante sana (-Thank you very much- in Swahili)!

De infectiologen (i.o.), HIV consulenten en virologen van het UMC St Radboud wil ik bedanken voor de gezellige, en vooral, leerzame HIV patiëntenbesprekingen.

De medewerkers van het Clinical Research Centre Nijmegen dank ik voor hun bijdrage aan de uitvoering van de onderzoeken in gezonde vrijwilligers (Hoofdstuk 3, 6 & 7).

Luuk Gras, ik dank je voor het verzamelen van de database gegevens voor Hoofdstuk 8. Reinout van Crevel, Martin Boeree, Kees Brinkman, Jan Prins en Job Juttman, jullie klinische blik was onmisbaar bij het tot stand komen van hoofdstuk 8.

De leden van de manuscriptcommissie, Prof. dr. de Groot, Prof. dr. Brouwers en Prof. dr. Lange, wil ik bedanken voor het bestuderen en beoordelen van dit proefschrift.

Lotte en Elke, ik vind het geweldig dat jullie als paranimfen achter mij willen staan. Lotte, ontmoet tijdens pianoles en vriendin vanaf de brugklas. Het feit dat je zelf in Afrika stage hebt gelopen, heeft waarschijnlijk bijgedragen aan je enthousiasme voor het onderzoek in dit proefschrift. Bedankt hiervoor! Elke, we zijn vriendinnen vanaf de introductie van de studie Farmacie. Ik heb enorm genoten van onze studententijd en vooral van de onderzoeksstage in Finland. Super dat ik als paranimf achter jou mocht staan afgelopen jaar!

Vrienden en familie, dank voor jullie interesse in mijn promotietraject. Het was altijd weer heerlijk om thuis te komen en van jullie te genieten!

Lieve Koen, mijn grote steun en toeverlaat, bedankt dat je me zo vaak naar het buitenland hebt laten gaan. Ik heb je gemist! Gelukkig ben je af en toe achter me aan gevlogen om samen op ontdekkingsreis te gaan. Ik vind je oprechte interesse voor de onderwerpen van mijn proefschrift heel bijzonder.

En als laatste natuurlijk, lieve Lenthe, bedankt voor het gezellige getrappel in mijn buik tijdens het afronden van dit proefschrift. Je maakt ons heel gelukkig!

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## Curriculum Vitae

Rafaëlla Francisca Anna L'homme werd geboren op 1 juli 1978 te Heerlen. Ze groeide op in Wijlre en behaalde in 1996 het V.W.O. diploma aan het Katholiek Gymnasium Rolduc te Kerkrade. Aansluitend werd begonnen met de studie Farmaceutische Wetenschappen aan de Universiteit Utrecht. Ter afsluiting van de doctoraal opleiding volgde ze een wetenschappelijke stage aan het Folkhälsan Research Centre te Helsinki (Finland). In januari 2001 behaalde ze het doctoraal examen. Ter afsluiting van de apothekers opleiding volgde ze een stage in het King Edward VIII ziekenhuis te Durban (Zuid-Afrika). Het apothekers examen werd behaald in februari 2003. Na een rondreis door Zuid-Amerika werkte ze van december 2003 tot en met juli 2004 als project apotheker bij Altrecht GGz te Den Dolder. In augustus 2004 begon ze met haar promotieonderzoek bij de afdeling Apotheek/Klinische Farmacie van het UMC St Radboud te Nijmegen. Rafaëlla is vriendin van Koen en moeder van Lenthe van den Broek.

